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A PRELIMINARY STUDY OF TEOSINTE IN ITS REGION OF ORIGIN

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Teosinte (Euchlaena mexicana Schrad.) is the closest known relative f maize, with which it readily hybridizes in nature. It has many charac-ers in common with maize, the two species differing most markedly in heir female inflorescences. Teosinte is a tall grass resembling maize. t produces many trapezoid and triangular black or brown hard seeds orne in spikes 4 to 12 cm. long. The plant is distributed from north Central Mexico south-eastward through Guatemala into Honduras. It oc-urs wild in semi-arid climates at altitudes from 2000 to 7000 feet. Its resent natural distribution is largely determined by climate and the de-tructive influence of different types of agriculture. Through the agency f man teosinte has been carried around the world in the tropics and in he southern fringe of the north temperate zone. In some localities it has ound some limited use as a forage crop, but apparently not as a cereal.

Although teosinte has long been known to the aboriginal people of Mexico and Central America, no positive evidence has been found that it was ever an important item in their diet. There is indirect evidence, however, that it was sometimes used for food, at least in times of scar-city and famine.

The distribution (7,20, 3, 14, 24), gross morphology (25, 26, 27), and cytogenetic relationship of teosinte to maize(1, 12, 17, 18), have been dis-cussed; but detailed studies of its functions, growth response, possible cultivation, and food value have received little attention. In this paper it is proposed to report new data relating to its distribution, growth, food value, and culture in Guatemala, its place of origin.

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The reader is referred to Kempton and Popenoe's (9) excellent pape for a comprehensive summary of the known distribution of teosinte pre vious to 1937. Rossignon (20) reported teosinte in 1869 from the vicinit of the pueblo of Santa Rosa in the department of Santa Rosa, Guatemala A search in this location by Collins (3) in 1931 in company with Jame Kempton and Wilson Popenoe failed to locate the plant. Collins says tha "it was learned that Dr. N. I. Vavilov had recently visited that distric on the same quest, and with the same negative results." This led to th inference that teosinte had disappeared from Guatemala. As a result Collins (3), in 1932, reported the rediscovery of teosinte in Guatemal by Dr. Wilson Popenoe and his wife who found it growing first alon the road from Jutiapa to Progreso in the department of Jutiapa i southeastern Guatemala, an area that drains southeast into the Pacifi Ocean.

That it probably was present in the Santa Rosa region when Collins an others made their explorations is indicated by the fact that J. R. Walli collected teosinte October 18, 1945, near the road between Cuilapa an Barberena, about five miles from Santa Rosa. In his report to the senio author covering a teosinte survey of the Jutiapa, Progreso and Lag Retana area, he wrote as follows: "The next day on the road to Barberen near Cuilapa, teosinte was found growing in abundance in a rice field The elevation of the field was about 3200 feet. The rice had been cut The following measurements were made of the teosinte. Height 10 feet diameter stalk 0.7 inches, leaves 20, leaf width 2.0 inches, side branche 5, tassel branches 16, seeds typical teosinte." Barberena is about fiv air miles from Santa Rosa and both pueblos are in the Rio de los Esclavo river basin. The Santa Rosa area is largely cattle country with comparatively little land under cultivation. Heavy grazing is destructive to teosinte. There can hardly be any doubt that Rossignon found the teosint that he sent to France in the Santa Rosa area. The accompanying outlin map (Fig. 1) shows the present known distribution in the Santa Ros region.

Kempton and Popenoe (9) also found it in this region at Lago Retana a small lake not far from Progreso. They reported teosinte growing dow to the water's edge. The senior author has visited the Jutiapa-Progres region and Lago Retana every year since 1945, except 1948, but at n time has teosinte been found growing near the water's edge and in swamp areas. It has only been observed on the dry banks not exposed to grazing back from the water. It may be surmised that Kempton and Popenoe (9 witnessed a condition brought about by a refilling of the lake following dry period. Lago Retana is a very shallow lake that periodically drie up. It was filled in 1946 and 1947, and dry in 1950 and 1951. Teosinte like corn, has none of the characteristics of a hydrophyte.

A very significant station was discovered by Standley (22) in 1949. H found teosinte in a wild state near the village of San Antonio de Padua i Honduras, fully 500 km. east of the most eastern station in Guatemala a Jutiapa. In this region teosinte was known as "maiz café" or "maiz silvestre." This station is of special interest because it is the mos southern and eastern known. Standley states further that "there are ver recent but still unverified reports of teosinte from the mountains of th

Fig. 1. The stations where teosinte has been collected in South East Guatemala with estimated distances from Santa Rosa, the original station established by Rossignon in 1869. Doubtless as further surveys are made, the number of stations will be increased.

department of Francisco Morazan, not far from Tegucigalpa, from the mountains of Copan and even from Nicaragua.

In 1949, the authors made a survey for teosinte in the department of Progreso in the watershed of the Rio Motagua that empties into the Carribean sea bordering Guatemala on the northeast. Teosinte was found growing wild near the pueblo of El Progreso (altitude 2500 to 3500 feet) in the valley of the Rio Agua Caliente that empties into the Rio Grande which feeds the Motagua. This station assumes much importance because it lies not in the Pacific Ocean watershed but in the Carribean. This means that teosinte may well be found throughout the area drained by the Rio Motagua extending northwest toward the San Antonio Huista region in Huehuetenango, some 150 km. away where teosinte occurs.

In September 1951, teosinte was found by Frank Smith and Marcial Barrios, employees of the Tropical Research Center, in the vicinity of

San Jeronimo (altitude about 1800 to 2000 feet) in the department of Jutiapa between 10 and 12 miles south of the pueblo of Jutiapa. This is the most southern station for teosinte in Guatemala. The area aroun San Jeronimo is largely pasture land with here and there a small milpa two acres or less. The teosinte grew among the grasses on the flat lar and on the rocky cliffs of the low mountain ridges. San Jeronimo is le than 25 miles from Santa Rosa where Rossignon collected teosinte in 186 There is reason to believe that teosinte is widely distributed in the are about San Jeronimo, thus placing teosinte in the environs of Santa Rosa.

In November 1951 teosinte, growing wild, was discovered at Jalotepequ in the department of Jalapa, also in southeastern Guatemala. Jalotepequ is situated in the valley of Rio San Jose in the watershed of the R Motagua that flows into the Carribean. This location is about half wa between Jutiapa-Progreso station and the El Progreso location to th north in the Department of Jalapa.

Kempton and Popenoe (9) in 1937 reported a new location for teosint in the Cuchumatanes mountains in the department of Huehuetenango. Teo sinte was found growing wild at 900 to 130 meters altitude in the vicinit of the pueblo of San Antonio Huista.

In 1944, Dr. George Goodman and the senior author also visited Sa Antonio Huista and confirmed Kempton's and Popenoe's record of teosint and at the same time extended the range into the valley of the Rio Azul.

Teosinte was common about San Antonio Huista but could hardly b said to cover thousands of acres as reported by Kempton and Popenoe, a least, such was not the case in 1946. It occurred along the road sides, i the corn fields, and in deserted milpas. In some of the fields of corn th teosinte was two feet tall, being cut with a machete rather than dug ou with the hoe. In other instances the fields were only infested with teosint along the borders of the milpas in stone and dirt fences. In the older de serted fields teosinte was gradually being shaded out by encroachin shrubs and other tall vegetation. The stand of teosinte was often dense i recently deserted fields but became much less common and scattered a the competition with other vegetation increased. The indications wer that teosinte needed open ground in order to flourish.

In a side trip from San Antonio to Santa Ana Huista (altitude 2700 feet) following the river down about 9 miles with altitude dropping from abo 4000 to 2700 feet, corn, early and late, was at first predominant, bu ceased to be the chief crop as we approached Santa Ana Huista. Coffe and other crops came into the picture. It is significant to note that teo sinte generally decreased in the wild state as we dropped down to Sant Ana Huista. Teosinte disappeared only when shaded out by taller plant such as shrubs and low trees. Several deserted milpas were observe having a dense stand of teosinte, but in the other places teosinte occurre only in isolated patches.

Kempton and Popenoe (9) mention that teosinte was the dominant plan in 1935 in the San Antonio area. This was not the case in 1944, except i the deserted milpas. A mixture of grasses, low trees and shrubs con stituted the dominant plant structure. They comprised the succession common to deserted milpas in the region. Competition and shading had great deal to do with the prevalence and distribution of teosinte.

As we left the valley of the Rio Huista and crossed the mountain ridg

en route to Jacaltenango in the valley of the Rio Azul, teosinte disappeared before corn at the higher elevations. From the top of the ridge down into the valley of the Rio Azul into the village of Jacaltenango, a distance of approximately five miles, no teosinte was observed although corn fields became common as we dropped down into the valley.

On a side trip from Jacaltenango down to Chitspaj, a distance of about 10 miles on the west side of the river, teosinte began to appear along the roadside about two miles from Jacaltenango. There were many deserted milpas on the low ridges around Chitspaj with teosinte in the same way as around San Antonio Huista. The abundance of teosinte in the vicinity of Chitsbaj rivaled that around San Antonio Huista. The stage of development was the same. We were told that it extended more than 10 miles down the river below Chitsbaj. The Rio Azul empties into the Rio Dolores which flows west into the Rio Nenton that empties in turn into the Rio San Gregorio in the State of Chiapas, Mexico. It is significant that Kempton and Popenoe (9) found little teosinte in the Jacaltenango region. This may well be because their expedition crossed over on the north side of the river going to Nenton through San Martin, while the stand of teosinte was on the south side down the river.

There was strong circumstantial evidence, too, that teosinte grew wild in the valley of the Rio Cuilco in south western Huehuetenango. Reports of the occurrence in this region have come to hand several times. The topography and climate of this area are favorable. Through the Intendente of the Municipalidad of Cuilco, it was learned that teosinte occurred in the Rio Cuilco Valley at elevations between 2979 and 3579 feet. The report of the Intendente Municipal follows: "Referring to a communication I received from the Senor Intendente Municipal de Huehuetenango, dated October 9, 1945, concerning whether or not the wild plant known as "milpa de rayo", "salic", is growing in wild state in this Municipio, I have the pleasure to report to you that after a diligent search, this plant occurs in this place under the name of "madre de maiz" or "madre de milpa." Therefore, the answers to the questionnaire you sent are as follows: There is an abundance, in the Municipio, of this plant. But the proprietors of the lands try to kill it to let the crops grow better. It is hard to state the area in which this plant occurs because it is exterminated when it grows. This plant occurs almost in the whole Municipio. Cuilco is nearly 1200 km. above the sea level and this plant can be found from 1200 to 1300 m. more or less. (signed) Intendente Municipal."

It may well be that teosinte occurs in several of the river valleys that lead into the Rio San Gregorio and Rio Grand in western Huehuetenango, but the reported presence of the plant needs to be confirmed.

Possible Early Uses of Teosinte

The records relating to the use of teosinte by aboriginal races because of their destruction by the Spanish conquistadors are fragmentary, especially so in the pre-Columbian period. Just what the situation was before corn became their staple food, remains largely unknown. These natives before they had corn must have had other plants which they used and that these persist today cannot be doubted; that teosinte was one of these plants is suggested by the present day custom of protecting volunteer

food plants in their patios and milpas. Teosinte is not the only vol unteer plant that is afforded this protection. There are many others suc as: Solanum nigrum L. used like spinach; species of Physalis used i sauces and cooked with meats; Phaseolus coccineus L. excellent whe cooked; Amaranthus species, the seeds of which are sometimes groun into meal (21); Sechium edule Swartz, of which fruit, roots and tips a cooked; Arracacia xanthorrhiza Bancroft, whose roots and leaf petiole are cooked; and others. This custom of affording protection to voluntee food plants is so well established that one may find such plants growing i door yards and among flowers. To destroy them is sometimes regarde as a bad omen. This protection is a custom of great antiquity. One ex planation given for protecting these volunteer food plants is that as foo they may be needed tomorrow. Even the descendants of the Mayan people employed to keep our trial grounds clean of weeds, frequently left stra volunteer food plants standing between the rows or in the rows of corn In some respects, customs rival written records in significance an authenticity.

Some of the above food plants may be little used today, as the grain c Amaranthus and the grain of teosinte, because other more desirable plant have replaced them. It is common practice in our own agriculture for th farmer to replace an unimproved variety with an improved. For example the flint corn varieties of the United States have been largely replaced b the softer and the more prolific dents. The open pollinated varieties o corn were replaced when hybrids became available, and the wilt suscep tible varieties of flax in the Dakotas largely disappeared with the comin of the new resistant varieties.

It is a common practice today for the people in eastern Guatemala an in El Salvador to plant grain sorghum with their corn as a safeguar against possible failure of the corn and consequent famine. The grai sorghum is not a preferred food and is only used alone or mixed with cor when there is a scarcity of corn. When corn is available, the sorghum i used only for pigs and chickens. The guarding of teosinte, coupled wit the meaning of the name,God grass, and its supposed use in early reli- gious ceremonies justifies the assumption that teosinte had a greate significance than just that of a plant resembling corn.

There is, however, some fragmentary evidence in the post-Columbia literature that teosinte had a place in the lives of the ancient people Hernandez (7) in 1790 records that an ounce of the ground grain was cure for dysentery. De Condolle (4), wrote concerning teosinte: "Probabl so unprotected a species was becoming extinct, more and more rare i some limited regions and was on the point of becoming extinct, when wandering tribe of savages, having perceived its nutritional qualities saved it from destruction." Rossignon (20) in a communication to France wrote as follows: "Je vous remets également quelques graines d'un espèce de Roseau ou de Totang indigène des parties chaudes, nommée Bocca-costa de la république de Guatemala, où l'on connait cette plante sous le nom de Téozinte; les habitants de Santa-Rosa (départment du meme nom, S.-O, de Guatemala), mangent les jeunes pousses, donnen les feuilles au bétail et se servent de la tige pour faire des cases, de haies des claies et meme des cannes." The eating of young shoots an inflorescences of various plants is a common practice in much of Centra

merica and Indonesia. The young ear shoots of corn are boiled or fried. is safe to assume that Rossignon referred to the young ear shoots.

That the descendants of the aboriginal people may in times of dis- ess have turned to teosinte, is well illustrated in an interview held ith Mrs. Josefa Cermeno, a woman 75 years old, who had lived all her fe in the pueblo of San Jeronimo in the department of Jutiapa in south- astern Guatemala. The pueblo is well back from the more densely pop- ated portion of the department of Jutiapa, in an unproductive area. She elated that her husband often planted teosinte in the corn rows so as to nprove the corn, and that when other corn was not at hand she made rtillas from the small ears of teosinte mixed with corn. Standley (23) eports that, in a station he located in Honduras, the natives roasted osinte as a substitute for coffee.

The high nutritive value of teosinte, discussed later in this paper, nds logic to the custom of protection and reverence with which teosinte as regarded.

Teosinte is better known as a forage plant than as a grain crop. It has een used as forage by the natives for a long time and is still used in ome regions. In the Progreso Jutiapa area the teosinte that volunteers n the used and unused milpas is not always considered as a worthless eed because as soon as the corn is harvested the cattle are allowed to asture the milpas. The cattle eat not only the green, but also the dried, illers and ears of teosinte. The value of teosinte in these areas is con- iderable because it affords feed for cattle during part of the dry season. ometimes wild teosinte is cut and tied into bundles and stored for feed or work oxen and cows. Teosinte is also used to a limited extent as a orage plant in the southern United States. The following green forage ields have been reported: Louisiana, 50 tons per acre; Georgia, 19; Mississippi, 22; and South Carolina, 22. Seed is sold for this purpose by several seed companies such as Reuter Seed Company of New Orleans, ouisiana, and other companies in southern United States.

MacMillan (10) reports that in Madras, India, teosinte was used for orage. On irrigated land one cutting yielded 20 tons per acre. Three cuttings in one season were estimated to yield 60 to 80 tons of forage, which was readily consumed by cattle and horses. Teosinte has been tried in other places in the tropics as in Burma, Indonesia, the Philippines, etc. without finding a place as a forage plant.

Unfortunately the trials that have been made of teosinte as a forage plant have been done without an understanding of its growth habits, i.e. its reaction to day length and the high nutritive value of the seed. The forage value has been based on the tonnage and nutritive value of the non-fruiting plant alone. No detailed studies have been made of its culture in relation to maximum production of green forage, silage, or grain. The high silage value of corn is not inherent in the plant before ear formation. It is the grain in its early stages of maturity that accounts for its high feed value. The same general principle would hold for teosinte. In any consideration of teosinte as forage and silage, it might be well to delay cutting until the fruiting spikes are well filled and in the hard dough stage because of the high feed value of the grain.

Some Food Values of Teosinte

The possibility of using teosinte as a cereal has not been explored i recent times. At present the plant is considered by some to be a worth less, weedy grass, interesting only because of its relation to corn. I 1926 Kempton stated: "We have no reason to believe that the grasse most nearly related ot maize were of the slightest value as a source c human food to the ancients. In the first place, their characteristics ar such as to render the extraction of the carbohydrates very laborious and to obtain seed in any quantity, an almost daily harvest would be required Secondly, other more promising wild plants were available but were neve adapted, and lastly there is no evidence that maize relatives were use for food even in time of scarcity." Mangelsdorf (12) also writes dispar agingly of teosinte as a food plant: "Like corn, it has tassels and ear borne separately, although its ears contain only five or six seeds, eac enclosed in a hard, bony shell, characteristics that make teosinte a mos unpromising food plant." In a later chapter it will be shown that these gen eral statements are not wholly in accord with recent experimental evi dence.

Observations of wild teosinte, the guarding of teosinte by natives an the use of the seeds by birds and other animals suggested the advisabilit of more adequately assessing its food value. A protein and fat analysis b Dr. B. Brimhall of the Service Laboratory of the Chemistry Departmen of the Iowa State College was made in January 1950. This showed 23 pe cent protein and 4.5 per cent fat. Further analyses were made of fiv different strains of teosinte in 1950 and 1951 in the laboratories of th Institute of Nutrition of Central America and Panama in Guatemala City Some of the data as published by Melhus, Aguirre and Schrimshaw (16) i shown in Table 1.

The data in Table 1 show that the hulled teosintes had a higher nitroge content than maize and higher also than wheat, oats, rice, and som beans. The tryptophan content was lower than that of maize, but methio nine was higher, in four samples from 0.45 to 0.58 per cent, and als higher than in rice, as reported by Kudra and Chowdhury (21).

Further studies were made of the protein and fat content of teosint grown in 1952 both wild or under cultivation. (See Table 2). Protein an fat were also determined in the outer and inner glumes and in grain pol ished either in a rice polisher or in a barley pearler.

The protein per cent ranged from 20.13 to 23.65 except in the sampl polished with the barley pearler which showed 18.75 per cent. The barle pearler reduced the size of the grain materially and removed many germs The grain polished in the rice polisher was not reduced in size as much a that run through the barley pearler, and the protein content was about tw per cent higher. Probably a barley pearler may be used to polish teosint by adjustment of the polishing surfaces.

The fat content varied from 2.26 to 4.43 per cent. Some of the fat wa lost when the grain was crushed or broken as was apparent by discoloratio of the white paper envelopes in which some of the samples were stored

The outer and inner glumes were, as might be anticipated, much lowe in protein and fat than the grain, and the outer glumes and rachises ha more protein and fat than the chaffy glumes.

TABLE 1

Chemical Composition of Teosinte Grain Compared with Maize

onstituents	Teosinte*		Maize**
	Grain With Hull	Grain Without Hull	
at	--	3.02	4.55
rude fiber	17.7	0.24	1.40
itrogen	1.47	3.48	1.54
sh	3.64	1.08	1.47
hosphorus	276.60	293.10	221.7
ron	6.20	4.69	2.44
hiamine	0.26	0.20	0.45
iboflavin	0.11	0.08	0.10
iacin	0.77	0.89	1.26
ethionine	--	0.52	0.16
ysine	--	0.37	0.29
ryptophan	--	0.044	0.048

he fat, crude fiber, nitrogen, ash, methionine, lysine, and tryptophan re expressed in terms of grams per cent. The others are expressed in illigrams per cent. All are calculated on the basis of ten per cent oisture.

Two samples of seed and four of teosinte grain were analysed, three from uatemala and one from Florida.

*Maize: A strain of flint corn, known as Tiquisate Golden Yellow, devel-ped by the Tropical Research Center and adapted to the coastal plain of uatemala.

Four individual plants of the collection 35-51, grown in 1952 and hand ulled, showed a variation of 2.27 per cent in protein and 1.32 in fat. The limited data suggest that considerable variation exists in the protein and fat in different strains of 35-51.

Latitude and altitude climates seem to have little effect on the protein and fat content. Antigua has a latitude of 14°+N. and central Florida, where 97-50 was grown, has about 26°N. The altitude of Tiquisate is 150 feet and Chalco, Mexico about 7500.

Percentages of Ten Amino Acids in Polished Teosinte

The quality of the protein as determined by amino acid content is as important as the quantity. Ten amino acids of the polished grain of teosinte were determined. The polishing was done with a rice polisher after the hull had been removed. The limited data of Table 3 indicate that the amino acid content of teosinte compares favorably with that of four other cereals. Teosinte grain presented a new source of cereal protein, comparatively rich in the essential amino acids.

TABLE 2*

Protein and Fat of Teosinte Grain and Glumes

No. Entry	Parent Seed Collected	Grown	Year Grown	Hulls Removed By	Protein Per Cent	Fat Per Cent	Remarks
35-51-31	Jutiapa, Guate.	Tiquisate	1952	hand	21.71	3.05	Cult.
35-51-14	" "	"	"	"	22.41	3.06	"
35-51-15	" "	"	"	"	20.13	2.43	"
35-51-16	" "	Antigua	"	"	21.93	3.75	"
35-51	" "	Tiquisate	"	machine	20.61	4.43	"
11-52[a]	Jutiapa, Guate.	Tiquisate	1952	machine	3.66	1.57	Cult.
12-52[b]	" "	"	"	"	2.23	0.95	"
97-50	Florida, U.S.A.	Florida	1949	hand	21.75	2.26	Cult.
223	Chalco, Mex.	Antigua	1948	"	23.00	4.26	"
35-51	Jutiapa, Guate.	"	1951	"	20.31	3.02	Wild
3-53	San Antonio, "	San Ant.	1952	"	23.65	3.53	"
35-51[c]	Jutiapa, Guate.	Tiquisate	1952	machine	20.78		Cult.
35-51[d]	" "	"	"	"	18.75		"

* The authors are indebted to the personnel of the Chemistry Department Service Laboratory of the Iowa State College for all the analyses, except entries 97-50, 223 and 35-51 (grown in 1951) which were taken from Melhus, Aguirre, and Schrimshaw (16). All calculations are on the basis of ten per cent moisture. a. The hulls comprise segments of the rachis and the outer glume. b. Inner chaffy glumes. c. The grain was polished in a rice polisher. d. A barley pearler was used for polishing.

TABLE 3*

Amino Acid Content of Polished Teosinte, Corn Meal, Oat Meal, Unpolished Rice, and Whole Grain of Teosinte

Amino Acid	Teosinte Polished Per Cent		Corn Meal Per Cent		Oat Meal Per Cent		Rice Unpolished Per Cent		Wheat Whole Grain Per Cent	
	in sample	in crude protein	in sample	in crude protein	in sample	in crude protein	in sample	in crude protein	in sample	in crude protein
Arginine	0.62	2.95	0.45	4.82	0.99	6.71	0.54	8.44	0.63	4.92
Histidine	0.48	2.28	0.24	2.57	0.31	2.10	0.14	2.19	0.31	2.42
Isoleucine	1.00	4.76	0.36	3.85	0.63	4.27	0.28	4.38	0.58	4.53
Leucine	4.10	19.52	1.11	11.89	1.14	7.72	0.51	7.98	0.91	7.11
Lysine	0.30	1.43	0.29	3.10	0.62	4.20	0.28	4.38	0.35	2.73
Methionine	0.46	2.19	0.21	2.25	0.25	1.69	0.14	2.19	0.22	1.72
Phenylalanine	1.60	7.62	0.46	4.93	0.78	2.28	0.31	4.85	0.70	5.47
Threonine	0.95	4.52	0.34	3.64	0.48	3.25	0.22	3.44	0.38	2.97
Tryptophan	0.05	0.38	0.08	0.90	0.23	1.56	0.098	1.53	0.19	1.48
Valine	1.10	5.24	0.50	5.36	0.86	5.83	0.40	6.26	0.64	5.00
Protein	21.00		9.10		14.76		6.39		12.80	

*The authors are indebted to Dr. Thomas L. Hurst and Miss Dorothy DeFontaine of the Analytical Service Laboratory, Department of Chemistry of Iowa State College, for the analyses of teosinte. The values for corn meal, oat meal, rice, and wheat were taken from Lyman, Carl M., and K. A. Kuiken (The amino acid composition of meats and some other foods, Texas Agricultural Experiment Station, Bul. 708, 1949).

Amount of Protein in Corn-Teosinte Hybrids and in Some Species of Maydeae

The high protein of teosinte raises the question of the possible increas in the protein content of maize through hybridization. Selfed lines of 2 three way hybrids, having one parent as teosinte, varied in protein con tent from 8.13 to 12.00 per cent. More should be known about the protei content of the other members of the tribe Maydeae. The only species c the tribe where the protein content has been determined are Zea mays (8 to 10 per cent), Euchlaena mexicana (20 to 23 per cent), E. perenni Hitchc. (25 per cent), and Coix lachryma-jobi L. (7.3 per cent). Othe species may have been assayed but no published records have been found The protein content of corn-teosinte hybrids and other species of Maydea needs further exploration.

Seed Production of Teosinte

No data are available on the potential seed-producing capacity of indi- vidual teosinte plants, either in the wild or under cultivation. Teosint has been known for over 160 years. During this time it has come unde the searching eyes of many plant scientists and farmers, none of whor have reported its yield of grain.

It is proposed here to record certain preliminary 1951 and 1952 dat on seed production. The yield of seed, both in the wild and cultivate plants, varies widely being influenced by factors as crowding, soil fertil- ity, time of planting, day-length (5.10), and climatic factors such a rainfall and temperature. Where the stand is dense, seed production i low, but when the plant has ample room, the number of seeds per plan may be high. For example, in a fence row in the Jutiapa region in 195 where there were 21 to 42 plants per quadrat (39 x 39 inches), the averag number of spikes per plant was six and the number of seeds ranged fron 8 to 28. No tillers or branches were present on these plants, which reduced the possible number of spikes. Tillers develop from latent buds just at the surface of the soil or below. Branches arise from nodal buds of the main axis or on tillers well above the crown of the plant. Where the stand was less dense than in the fence row, both tillering and spike formation were more evident, but less extensive than in cultivated teo- sinte. Weatherwax (25) has reported from 10 to 60 branches and some- times more on wild plants.

In a cultivated plot planted May 28, 1951, at Antigua, using a Mexica variety, where the seeds were planted 8 to 12 inches apart in three foo rows, seed production per plant varied from 17 to 187 grams. The plants were dwarfed incident to the difference in latitude (climate) of Mexico (abou 20° N. Lat.) and Guatemala (about 14° N. Lat.) which difference sharpl influenced the vegetative and reproductive stages.

On the other hand, 32 plants spaced three feet apart on the Antigu plots, using adapted Guatemalan seed, averaged 11 tillers, 27 branches 237 spikes, and 11 ounces of seed. A similar response occurred i Tiquisate (150 feet altitude), as shown by the following counts. Plant were selected at random from a plot 237 x 308 feet, planted August 2, an harvested 119 days later. The rows were spaced 3 feet and the plant

Fig. 2. An individual plant of teosinte planted August 21, 1952 and harvested 103 days later. It had 12 tillers, 36 branches, 229 fruiting nodes, 10 ounces of seed, and 4.3 ounces of grain. The height was $11\frac{1}{2}$ feet. Tiquisate, Guatemala.

drilled in the rows. The per plant yield of five plants was 16.0, 15.6 4.0, 3.8, and 11.6 ounces, respectively. Six other plants counted in a adjoining plot of the same size and spaced as above, averaged 12. ounces of seed (see Fig. 2). These data on individual plants, though limited, indicate that in cultivation a plant may produce from 3.8 to 1 ounces of seed.

The results obtained on plot yields in 1951 are interesting. Two varieties were planted July 27 in six rows, each 100 feet long. The seed plots were from the Jutiapa area in Guatemala, (1-51) and from Florida (99-50). The seeds were drilled thick (one seed every two to four inches) in rows three feet apart. The stand was definitely too dense The crop was harvested 120 days after planting and the weight of seed determined. The Florida variety was shorter and matured about sever days earlier than the Guatemalan. The seed of 97-50 was small and brown and that of 1-51 large and black. The difference in the latitud climate of Florida and Tiquisate probably was a contributing factor to the difference in the response of the two strains of teosinte (5, 10).

The crop was harvested by cutting the plants at the ground level and flailing on a canvas. In this process some of the seed was unavoidabl lost. There was also considerable shattering before the crop was harvested. No attempt was made to gather and weigh the seed from individual plants nor to count the panicles and spikes, but the weight of the harvested seed from six rows was 57½ pounds, nearly a bushel from about 1/24th o an acre (a bushel of teosinte seed weighs 60 pounds). This preliminar trial did not result in accurate data on yield.

In 1952, four half-acre plots were planted at Tiquisate on land that ha been cleared three years previously. The purpose of this preliminar field experiment was twofold: first, to gain some information about yield and, second, about mechanical harvesting of the crop with a combine The plots were large enough to obtain data on mechanical harvesting.

Two crops of corn had been grown on the land. The first half of the third year the land was allowed to grow up to weeds. It was plowed in late July and fitted for teosinte. The excessive amount of organic matter incident to the weed growth left the surface ground irregular. Two low spots in the plots did not drain well in periods of heavy rain.

The seed was planted (drilled) with a Planet Junior garden planter starting August 2, but not finished until August 21 because of a heavy rain that interrupted the plantings. Not all of the seed grew due to the state of tilth of the land the planter used and a four inch rain that fell, after two of the plots (A and B) had been planted. As a result, the stand was uneven with some bare spots. In two of the plots the rows were 42 inches apart and in the other two 21. Six to seven pounds of seed were used per acre on each of the former plots and 12 to 14 on the latter.

The plots were cleaned (hand hoed) once when the plants were 6 to 8 inches tall. Later when the plants were about two feet tall, they were machine cultivated. The plots received no other cultivation and were free of weeds at harvest time, December 2, because of the shading caused by the profuse tillering and branching of the teosinte.

The plants grew from 10 to 13 feet tall and matured seed, ready for harvest, in 119 days (see Fig. 3). There was no lodging except in the we areas. The crop sustained little injury from plant pathogens and insec

Fig. 3. The development of teosinte 90 days after planting on the Tiquisate plots (150 ft. Alt.). The plants were 10 to 13 feet tall and the fruiting spikes were fertilized and beginning to mature. Note the tillering and branching. The rows were 42 inches apart. There was no lodging.

pests. The yields obtained are shown in Table 4. There was a wid variation in the yield of seed in the four plots. The lowest yield was 1065 and the highest 1700 pounds per acre, a spread of 635 pounds. This variation was largely due to stand and differences in the growing conditions. The data indicate that 1600 to 1700 pounds of seed may be produced or one acre (see Fig. 4).

TABLE 4*

Seed Yield of Teosinte on Four Half-acre Plots at Tiquisate 1952

No. Plot	Planting Date	Row Spacing (inches)	Seeding Rate (lbs.)	Yield Per Acre (lbs.)	Mean Moisture (%)
A	8-2	42	6-7	1156	13.9
B	8-2	21	12-14	1065	15.8
G	8-21	21	12-14	1700	18.9
H	8-21	42	6-7	1637	14.9

*The seed was spread on a canvas and allowed to dry for two days. The moisture determinations are the means of three different samples. They were made with an American All-Crop Moisture tester.

The harvesting of the four plots was unduly long (20 hours) because o necessary time lost in adjusting the combine (see Fig. 5). The height of the large number of tillers and branches made it impossible to cut more than one row at a time. Although a stubble of 40 inches was left, the tillers were so long that some of the tassel ends fell on the hood covering the cylinder of the John Deere No. 12A combine. To overcome this difficulty partially, the reel was removed, and a worker with a stick steere the long tillers into the cylinder of the machine. However, even this precaution failed to prevent some loss of tillers. This loss decreased th measured yield; how much was not determined.

The threshing of the grain from fruiting panicles was quite thorough, and all of the seed fell through the separating sieves into the seed elevator. There was some shattering of the earliest maturing plants befor harvest, and some seed was eaten or shattered by birds, such as specie of Quiscalus (known in Spanish as sanate) and Conuropsis (parakeets).

Although this field trial failed to supply accurate data on yield and mechanical harvesting, it did indicate the response of teosinte to cultura practices in a tropical climate. Randomized and replicated experiment will need to be made on rates of seeding, row spacing, and methods o planting before yield can be determined accurately. The results show that 1700 pounds of seed may be produced per acre, and that the crop ca be harvested mechanically.

Hulling and Processing of the Grain

Removing the hulls is not a formidable problem with either primitiv or present day machinery. It is true that it is not as simple as separatin the grains of corn from the husks and cobs, which may well have ha much to do with making teosinte a very minor food plant by the natives

'ig. 4. Harvested just as it came from the combine to the drying floor. 'he seed was relatively clean and dry (16.9 per cent moisture).

Fig. 5. Harvesting teosinte mechanically. A rear view of a John Deere No. 12A used to cut and thresh teosinte. Note the 40 inch stubble. The crop was made in 103 to 119 days after seeding.

That teosinte is more difficult to hull than the old world cereals, barley, oats, wheat, and rice, is also true. However, the hulling of teosinte car be done with the primitive tools in use today in some places in Latir America and in Southeast Asia. The rough rice is hulled and corn mashe by shaping a wooden stick to work up and down in a stump or block o wood, actually a mortar and pestle. Too, the early indigenous people o this hemisphere had primitive stone tools such as mortars. Teosinte car be hulled in the same way as rough rice is hulled and as corn is mashed.

The seed of teosinte consists of a segment of the rachis and a harc glume covering the kernel embedded in an alveolus in the base of the rachis segment. Removing the kernels from these vegetative structure was not as difficult as anticipated. The grain was readily removed fron the hull by passing the seed through a small feed mill or rice huller. A Clinton and Company feed mill, pulled by a one-half horse power engine removed the hulls with ease without breaking the grain seriously (see Fig. 6). Best results have been obtained by grading the seed before running it through the feed mill so that the seeds are of the same size. Grading makes it possible to adjust the grinding discs close enough to brea away the hull from the grain and leave most of it whole.

The hulls and grain can be quite well separated with appropriat sieves in a Clipper 2-B Special Cleaner, such as made by A. T. Ferrel and Company, Saginaw, Michigan, or a Hart Carter disc machine. Als aspirators and gravity cleaners facilitate separation of the grain.

It was also possible to remove the hull and to polish the grain with rice huller (No. 1831), such as made by George S. Squier Manufacturin Company, Buffalo, N. Y. The hulling was done by a ridged cylinder operating against a knife. From the hulling chamber the grain drops int the polishing compartment. The polishing was done by a series of brushes The hulling was satisfactory, but the polishing was too severe (see Fig 7). Too much of the grain was removed, materially reducing its size The Lewis C. Grant Company huller of Kirkcaldy, Scotland, also hulle teosinte well. It consists of two flat stone discs working against on another. The polishing was done by another machine made by the sam company. Using the above equipment on 532 pounds of teosinte seed there was produced 37 per cent hulled and polished grain. Here again wa a sharp reduction in the size of the polished grain over the unpolished Of course, part of this was the coat of the kernel and the rest part of th grain. The kernels of teosinte are more spherical than the cylindrica rice kernels, a fact which probably accounted for the loss. Further adjustments in the polishing stones should decrease this loss. Another lo of 410 pounds of seed was hulled in a stone disc rice huller made b Koerber and Naumann of Hamburg, Germany. This machine yielded 40. per cent grain from 410 pounds of seed--18 per cent of which was partially polished.

The Proportion of Hull and Grain of Teosinte Seed

The proportion of hull and grain in the seeds assumes much importanc if teosinte is to find a place as a cereal. There is considerable variatio in the size and weight of the seeds and the grain. The spikes taper s that the terminal seeds are smaller than those in the middle of the spike

'ig. 6. A Clinton Feed Grinder and a small half horse gas engine used ɔ hull and grind teosinte seed and grain.

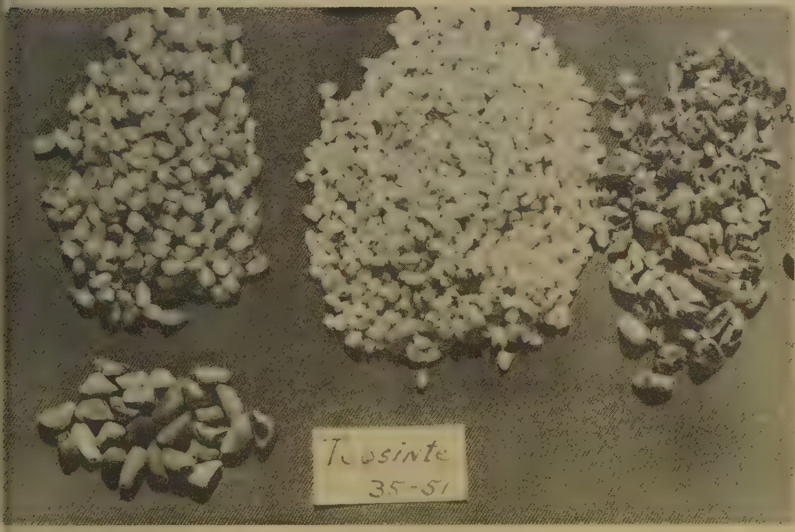

Fig. 7. The teosinte grain is covered with a hard hull. Hulled grain is ıt the upper left and the empty hulls at the right. The polished grain is in he middle pile. The unhulled grain is at the bottom left. The hulled grain constitutes about 40 to 50 per cent of the seed. The grains are ıbout 1/3 larger than wheat grains (Thatcher variety.)

Under favorable conditions a spike comprises from 10 to 14 seeds. Plants growing wild or under crowded field conditions may mature only three to eight seeds as has been described by Weatherwax (26), but there are no available data on the proportion of hull and grain.

Some light has been shed on this problem by hulling seed by hand and weighing the seed, hull, and grain separately. The data of Table 5 show the weights of seed, hull, and grain of five different lots.

TABLE 5*

Weights of Seed, Hull and Grain in Teosinte

No. of Seed Lot	Altitude (feet)	Seed Source	Seed (gms.)	Hulls (gms.)	Grain (gms.)	Grain Per cent
79-51	2500	Jutiapa, Guate.	9.2	5.2	3.9	42
35-51A	4953	Antigua, "	9.5	5.5	3.8	40
35-51A	50	Cuyuta, "	7.1	3.8	3.2	45
1-52A	7500	Chalco, Mexico	12.5	7.4	4.9	40
97-50	low	Florida, U.S.A.	8.5	4.7	3.8	45
TOTALS:			46.8	26.8	19.6	41.6**

*The seed of 79-51 was collected from wild teosinte. The 100 seeds were chosen at random from 10 to 50 pound lots. Immature empty seeds, usually dull white, were not included in the 100 seed lots.

**Mean.

The weight of hull and grain varied as shown above. This might be expected since the seed was produced in five different altitude climates.

In another series, weights were made involving Guatemalan seed (35-51) and seed grown at Chalco, Mexico (1-52A). The seed was thoroughly dried at 27° C. for six days. The following weights were obtained:

TABLE 6

Weights of Seed, Hull and Grain in Teosinte

Guatemalan Strain (35-51)						Mexican Strain (1-52A)		
Random Sample			Large Seeds			Random Sample		
Seed	Hull	Grain	Seed	Hull	Grain	Seed	Hull	Grain
9.6	6.1	3.5	14.7	7.1	7.5	10.0	5.3	4.7
8.7	4.9	3.8	15.2	7.7	7.4	9.0	5.1	3.9
9.4	5.2	4.2	15.8	7.8	7.9	9.7	5.6	4.1
9.8	5.2	4.6	15.7	7.5	8.1	9.5	5.3	4.2
8.5	4.8	3.7	13.8	6.7	7.0	9.5	5.5	4.0
Mean per cent Grain:		42.4			50.4			44.0

The data on percentage of grain seem to range from about 40 to 50 er cent. The larger seeds yielded the highest proportion of grain to ull. It would seem that in any production program one objective might ell be the development of large seeded strains. The proportion of hull grain is higher in teosinte than in rough rice (20 to 25 per cent hull) nd oats (25 to 40 per cent). The kernels of teosinte were larger than heat. The dry grain was harder than that of corn or wheat.

The Grain as a Food

The same feed mill that was used for breaking away the hulls was employed to grind the grain. By adjusting the grinding discs close together nd running the grain through the mill five times, it was possible to seure a coarse flour. By sieving this through miller's bolting cloth, a ine grade of flour was obtained. There is every reason to believe that a lour mill would be much more efficient than the Clinton feed grinder. Iowever, it did serve to supply flour for baking and cooking experiments.

The whole grain softens when boiled in salted water for one hour in a louble boiler or for a shorter time in a pressure cooker. The grain absorbs water and swells to twice the size of the dry grain, resembling a ooked breakfast food. The flavor is not that of corn or cracked steamed wheat. Teosinte grain has a mild nutty flavor and a somewhat granular exture, differing from any other cereal. The cooked grain has also been rolled into thin flakes and baked on a thin slab of steel forming dry flakes similar to some dry breakfast foods. They were light brown, crisp, and distinct in flavor from any other dry breakfast food tried.

Equal portions of corn and teosinte grain have been made into tortillas and pishtones, a form of tamale, of good texture and quality (see Fig. 8). The cooked grain was added to the corn and ground on a metate (the stone on which the corn is ground by the Indians). Teosinte grain alone formed a somewhat sticky masa (the corn mass ready to bake) which was not as easily formed into thin cakes as the masa consisting of half corn and half teosinte. Equal parts of wheat and teosinte flour were used for muffins (see Fig. 9). The palatability was as desirable and tasty as that of muffins made from wheat flour alone. The color was a bit darker due to the teosinte flour being an off color white and to the brown seed coat of the grain. If the latter is removed, as is done in making wheat flour, teosinte flour is nearly as white as that of wheat. The coarsely ground grain of teosinte has been made into bread, using baking powder in much the same way as in making corn bread. These preliminary tests, involving the use of whole grain and coarse and fine flour, indicate that the cooked and baked products rival wheat or corn products in palatability.

There are many possible uses of the grain of teosinte if the nutritive value proves in further chemical and biological assays to be as good as preliminary data indicate. The food industry may well find an outlet for products made from teosinte grain because of its high protein.

The grain may find a place where protein is needed in increased amounts, or to balance diets where people are largely dependent on a single cereal as corn or rice. Tortillas made of corn alone constitute the chief food of 85 per cent of the inhabitants of Guatemala and some other Latin American countries. In such cases tortillas may comprise

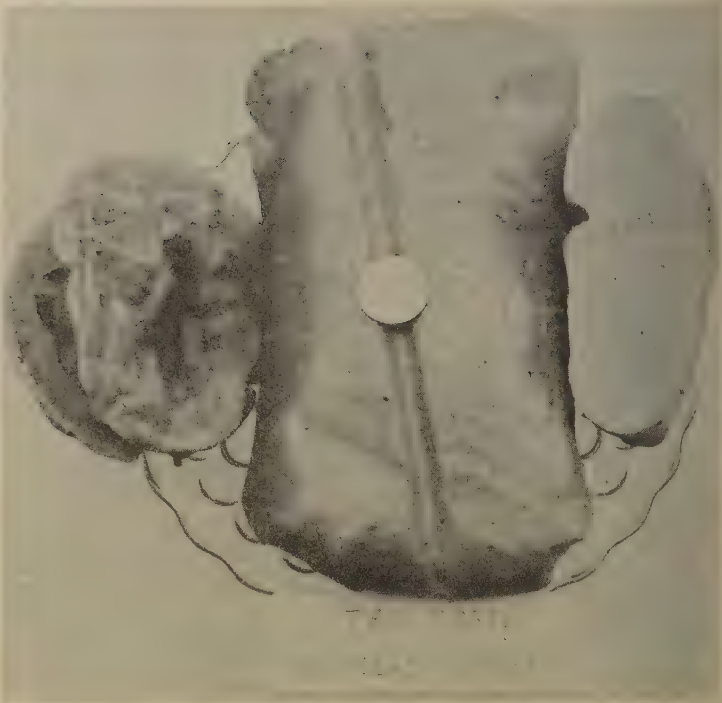

Fig. 8. A pishton (large tamale) underneath the five cent piece, two tortillas at the left and a cross section of the pishton. These were made from equal parts of teosinte and corn. Antigua, Guatemala, 1952.

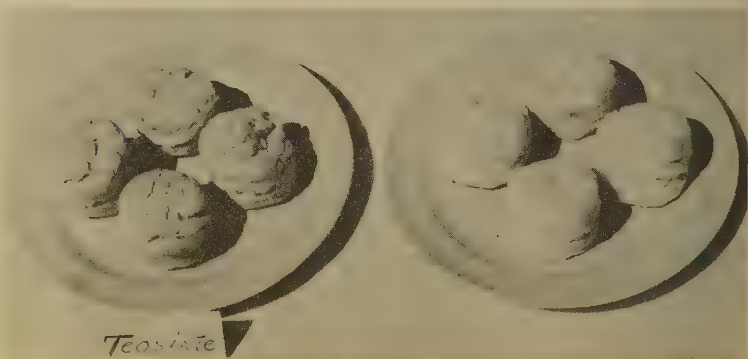

Fig. 9. Teosinte muffins were made using 3/4 teosinte and 1/4 wheat flour. The hot muffins were bland and had a texture like the wheat muffins. The color of the teosinte muffins was darker than the wheat because the whole grain was milled. Antigua, Guatemala, 1952.

0 to 95 per cent of the diet, and it is well known that the protein of corn eaves much to be desired in nutritive value. A similar condition prevails n other areas of the world, as southeast Asia, where rice is the main ood, also low in protein. The addition of a cheap bland cereal protein igh in nutritive value, that can be grown in the tropics would provide a imple method of improving the diets of the underprivileged. Whether eosinte can play this role and others that might be mentioned, is only artially answered in this paper.

Disease and Insect Pests

Teosinte, growing in its native habitat, is not seriously attacked by lant pathogens or insect pests. At no time in the past eight years has he senior author observed serious injury caused by fungus pests in native tands of teosinte. This may be due to the environment and growth habit f the plant in the wild. The plants in their native habitat are usually cattered, dwarfed, and in mixed plant populations. As stated earlier, he most favorable climatic zone for teosinte in Guatemala lies between 000 and 5000 feet where the rainfall ranges from 20 to 60 inches annu- lly. These environmental conditions and growth habits may be unfavor- ble to most plant pathogens and insect pests attacking teosinte. The ollowing fungi have been found on native teosinte: two smuts, Ustilago eae (Peckm.) Unger, and Sphacelotheca reiliana (Kuchu) Clint. (see Fig. 0); three rusts, Puccinia sorghi Schw., P. polysora Underw., and Angiospora zeae Mains; Helminthosporium turcicum Pass.; Phyllachora naydis Maubl., and a virus disease that may be the same as the corn tunt disease. None of these plant pathogens have become prevalent and lestructive on our experimental grounds in either small (10 hill rows) or larger plots comprising 1/10 acre of more. Puccinia sorghi is com- non on seedlings in fall but not on spring plantings. Helminthosporium urcicum is prevalent on Mexican strains of teosinte grown in Antigua when planted in March and April. Guatemalan strains, on the other hand, suffer little injury.

Three types of insect damage have been observed on teosinte in the wild, namely: Maize maggot injury, rootworm and stem borer injury, caused respectively by Euxesta major V. d. Wulp., Diabrotica and Diatraea species. In native stands these pests cause comparatively little injury, but when teosinte was grown adjoining corn, as on our corn plots at Antigua, they cause some damage. Maize maggot injury became preva- lent and destructive on seedlings in 1951 and 1952 when the seed was planted in July, August, and September on the Antigua trial grounds. The symptoms on the seedlings were much like those occurring on corn (6, 14) (see Fig. 12). Early plantings in March escaped injury. Maize maggot injury did not become prevalent on teosinte at Tiquisate (150 feet altitude) although the adult flies (Euxesta major) did occur. It is believed that the teosinte seedlings grow out of the seedling stage so quickly that the host escapes injury in the low coast region. On the other hand, at Antigua (4953 feet altitude) where the temperature is lower than at Tiquisate, the seedlings grow more slowly, affording the larvae a longer time to feed on the seedlings.

Rootworm injury was more common on teosinte planted in May and

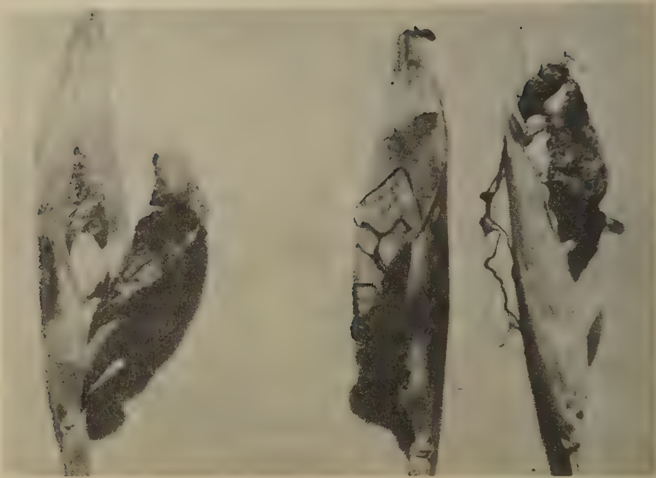

Fig. 10. Kernel smut (Sphacelotheca reiliana) of teosinte. This is rare c
wild and cultivated teosinte.

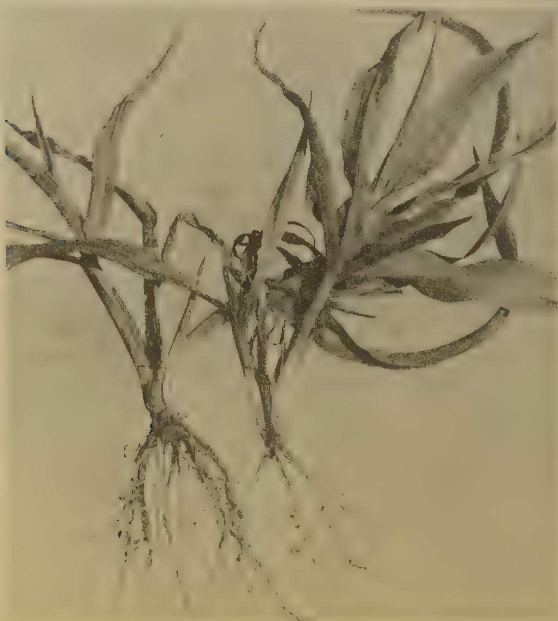

Fig. 11. Two teosinte plants injured by the maize maggot (Euxesta majc
V.d.Wulp). Note that the primary axis was killed. Adventitious bu(
gave rise to new tillers. Antigua Trial Grounds. December 1952.

une than on that planted in March in Antigua. Little or no rootworm in-ury occurred on teosinte planted in August in the lowlands at Tiquisate. tem borers were more common on teosinte planted in Antigua than ob-erved on wild plants in the Jutiapa region in 1950 and 1951. However, he damage caused was of little importance.

The rice weevil and grain moths (Sitophillus oryzae Linné and Sitotroga erestella Linné) may cause heavy losses to corn in the field and in stor-ge. They constitute the most serious insect pests of corn in the tropics. ry teosinte seeds, on the other hand, have not been observed to be nfested. However, these weevils have been observed feeding on fruiting pikes in the milk stage in the field, especially where the spikes were njured by birds. The seed integuments serve as mechanical barriers.

SUMMARY

Available teosinte distribution data tend to show that it is found in solated areas between 2000 and 5000 feet throughout the highlands of Guatemala. It is unknown in the low tropics and in mountainous climates. Plant successions made up of low shrubs are destructive to teosinte. This plant is better adapted to primitive cultural methods than is corn and persists because of its growth characteristics and integuments.

The native green plants are relished by horses, sheep, and cattle, and he seeds by wild birds, chickens, rodents, and peccary. The cultivated and deserted milpas are pastured after the corn harvest.

There is some evidence that descendants of the Mayas used teosinte as food in times of distress. The custom of guarding the plants suggests that the early indigenous people knew its food value.

Statements in the literature implying that the hardness of the seeds made them useless were not based on experimental evidence. The sepa-ration of the grain and hull can be done with primitive tools and modern machines.

The grain of teosinte is richer in protein than is corn, rice, or wheat. It has 18.75 to 23.65 per cent protein and 2.26 to 4.43 fat. The percent-age of methionine is high and of tryptophan low, as shown in tables 1, 2, and 3. Teosinte compares favorably in 10 essential amino acids with corn meal, oat meal, unpolished rice, and whole wheat grain.

Flour made from the grain is bland in flavor. Pishtones, muffins, and breakfast cereals have been made from the grain and were found to be palatable.

Little is known about crop yields of teosinte grain. Individual plants may produce from 3.8 to 16 ounces. In a preliminary field trial on one-half acre plots, the yield of seed ranged from 1065 to 1700 pounds or 17.7 to 28.3 bushels per acre (bushel weight: 60 pounds). The percentage of grain from hand hulled seed was 40 to 50 per cent. In a machine hulling experiment, the percentage of hulled grain was 40.3. The grain is larger and harder than that of wheat.

Teosinte is a host to most of the plant pathogens and insect pests that attack corn in Guatemala, but none of them have been observed to be as destructive on teosinte as on corn. The mature seeds of teosinte have not been observed infested with the rice weevil (Sitophilus oryzae) or grain moth (Sitotroga cerestella) so destructive to corn in the tropics.

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APPARATUS FOR EXTRACTION OF MICROQUANTITIES OF LIPIDS[1]

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Extraction apparatuses for small amounts of biological material ave been designed by Erdős and Pollak (1) and Hsiao (2). The set-up of rdős and Pollak consists of a perforated funnel held by conical notches n the neck of a Kjeldahl-like flask; solvent, heated in a water bath, cools n a four-ball condenser fitted into the flask by a ground joint and drips nto the sample in the funnel. Extraction is complete within three hours nd differs from macroextraction results by 0.75 per cent. Hsiao's appaatus consists of a 100 ml. Kjeldahl flask and a "cold-finger" condenser vhich closes the top of the flask. A six centimeter section of glass tubing vith a sintered-glass plate bottom is suspended in a perforated insect vial rom the bottom of the condenser by means of a glass rod. Alcohol in the lask is evaporated by a microflame, condenses on the "cold-finger", and lrips on the sample in the section of glass tubing. Ten minute extraction ives consistent results, and the amount of lipid is not less than that exracted by a macro-Soxhlet apparatus in six hours.

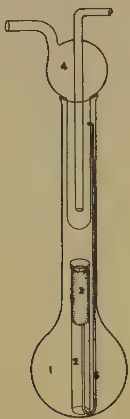

Fig. 1

[1] Journal Paper No. J-2307 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1102.

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A simplified microextractor for an insect lipid investigation wa designed from materials obtainable in almost any chemistry laboratory The extractor is shown in Fig. 1; it proved to be remarkably quick i efficient extracting. It consists of: (1) a 300 ml. Kjeldahl flask; (2) glas tubing with inside diameter of 10 to 11 mm. and rimmed at the upper end (3) a 10 x 50 mm. extraction thimble which fits tightly into (2) or rest against (5); (5) a glass (or metal) rod about 2 mm. in diameter with short hook at the upper end and a long hook at the lower end; and (4) "cold-finger" condenser which closes the top of (1).

Insect material, to be extracted, was ground in a beaker with a smal amount of ether-extracted sea sand by means of a heavy glass stirring ro and transferred quantitatively to the extraction thimble. The flask wa charged with approximately 20 ml. of solvent (ethyl alcohol-diethyl ethe 3:1) and heated in a sand bath. Extraction time was started when drops o condensed solvent began to fall from the condenser into the thimble Minor adjustments in the position of the condenser and thimble were mad so that condensed solvent fell into the extraction thimble and not onto th rim of the glass tube. At the end of the extraction period, the condense was removed and the extractor allowed to cool. Approximately 5 ml. o fresh solvent was poured onto the sample within the thimble, and the ro was hooked over the rim of the flask for a few minutes to permit drainage. Later, this rod along with the glass tube and the thimble were remove from the flask. The extraction fluid, along with at least two solven rinses of 5 ml. each, was transferred to a volumetric flask. In event a second extraction was to be carried out on the same sample, the flas was recharged with solvent and the extraction repeated.

Determination of the alcohol-ether extract by the oxidation proces indicated that extraction was complete at the end of 30 minutes, provide the solvent was changed at the midpoint of this period. A 30 minute, onc recharged, extraction removed an amount of alcohol-ether solubles no less than that extracted by the Soxhlet apparatus in 12 hours.

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EFFECT OF SUMMER MULCHES ON YIELDS OF EVERBEARING STRAWBERRIES, SOIL TEMPERATURE AND SOIL MOISTURE[1]

E. L. Denisen, R. H. Shaw, and B. F. Vance[2]

Everbearing strawberries are generally unproductive under Midwest conditions when grown in matted rows. Judkins (2) obtained great increases in yields of several everbearing varieties with close spacing of the plants, continuous runner removal during the entire season, and a sawdust mulch. Using similar planting plans, Denisen, Crandall and Doll (1) also increased the productiveness of everbearers by pruning off all runners and using either chopped corncobs or sawdust as the summer mulch. In the experiments of both Judkins (2) and Denisen et al. (1), the customary matted row system with no mulch was used as the check. With the matted row system, a single row of parent plants was allowed to form runners and establish new plants in a bed 24 to 30 inches in width. With the close spacing of plants in the mulched treatments, more plants per area were required since the runners were controlled. Consequently, the effects of runner removal and mulches in increasing yields could not be differentiated. The following report concerns an investigation on the relative importance of mulches and runner removal on yields of everbearing strawberries and the influence of mulches on soil temperature and moisture.

PROCEDURE

Plants of the Superfection everbearing strawberry were set out in beds in April, 1952. There were five treatments randomized in each of the six replications of the experiment. Four of the treatments were planted according to the three-row system with rows 12 inches apart and plants 12 inches apart within the rows. The other treatment consisted of the matted row system with plants spaced 12 inches apart in a single row. Plots were 20 feet long and there was a distance of 4 feet between the centers of adjacent plots. These spacings left areas for paths along the boundaries of adjacent plots.

Blossoms were removed from the plants of all treatments until June 20, and runners were removed from all three-row plots throughout the season. Six weeks after planting, all plots were given a thorough cultivation, and the summer mulches of sawdust, chopped corncobs and oat straw

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[1] Journal Paper No. J-2273 of the Iowa Agricultural Experiment Station, Ames, Iowa. Projects 1054 and 996.

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were applied at random on three-row plots in each replication. Th depth of mulch for each of the mulched treatments was 1 to $1\frac{1}{2}$ inches fo sawdust, $1\frac{1}{2}$ to 2 inches for chopped corncobs, and 2 to $2\frac{1}{2}$ inches for oa straw. The depths were fixed on the basis of the relative rates of decom position of the three materials. The matted row plot and one of the three row plots in each replication were not mulched and were considered a check treatments.

Soil moisture determinations were made for all treatments in thre replications on July 29 after two weeks with no precipitation and on Sep tember 27 after four weeks with no precipitation. Soil samples were col lected at 1 inch and 3 inch depths at three locations in each plot. A 15/1 inch cork borer was used at each location to extract a plug of soil. Th three soil plugs from each treatment at each depth were combined in soi moisture drying cans to make composite samples. The soil samples wer oven-dried at 104° C. for a minimum of 18 hours. All weight measure ments were determined to 1/1000 grams on a chain-o-matic balance.

Soil temperatures were measured in late July and early August usin a Brown recording potentiometer and copper constantan thermocouples The potentiometer recorded 16 different temperatures in 80 seconds. Ob servations were taken at 1 and 3 inches at frequent intervals so that th diurnal temperature curve could be plotted. During August and earl September temperature readings were taken at 4 p.m. with standar mercurial thermometers. The above ground portion of the thermometer was covered with aluminum foil to protect the thermometers from radia tion. In Fig. 1, the experimental plot set-up is pictured, showing th plots, thermometers and recording potentiometer.

Fig. 1. Experimental plot set-up. The plots, left to right in the foreground are: portion of the matted row, corn cob mulch, straw mulch, sawdust mulch, and unmulched. Location a. designates the recording potentiometer, b. thermocouples leading to the plots, and c. mercurial thermometers.

Yields of berries of marketable size were recorded throughout th picking season. At the end of the growing season, strawberry plants fror each treatment in two replications were dug and the soil washed from th

ɔots for root development comparisons under the various treatments.

RESULTS

During the early part of the growing season the plants became well ɜtablished; the matted row sent out numerous runners, and plants of the ɹree-row system developed large crowns and leaf areas. Following the ɴd of the blossom removal period (June 30), the plants started to set ·uit and the summer harvest was begun on July 28. A summary of the ields for the entire season is shown in Table 1.

TABLE 1

Late Summer Yields of Superfection Everbearing Strawberries Under Various Mulching and Spacing Systems

pacing and mulching treatments	Quarts of berries of marketable size per 100 feet of row[1]
ɬatted row, no mulch	3.5
-row, corncob mulch	25.7
-row, straw mulch	19.2
-row, sawdust mulch	31.6
-row, no mulch	22.4

[1] Level required for significance at .05 = 4.7 qts. at .01 = 6.5 qts.

ʻhe three row treatments all produced significantly higher yields at the 01 level than the matted row. Among the three-row treatments the plot ɴulched with sawdust were most productive followed, in order, by corn-·ob mulch, no mulch, and straw mulch.

Soil moisture percentages were low for all treatments as indicated in ʻable 2. Since both dates of sampling followed periods of dry weather, t seems likely that moisture was so critical that plants of all plots had ısed most of the available moisture.

TABLE 2

Average Soil Moisture Percentages, 1 and 3 Inch Depths, at Two Dates of Sampling

Spacing and mulching treatments	July 29 [1]		Sept. 27[2]	
	1" depth	3" depth	1" depth	3" depth
Matted row, no mulch	11.3	13.5	11.4	12.1
3-row, corncob mulch	12.9	14.4	12.1	13.3
3-row, straw mulch	13.2	13.3	12.8	12.9
3-row, sawdust mulch	13.0	14.1	12.4	13.4
3-row, no mulch	8.3	11.0	9.9	11.0
Mean, all treatments	11.7	13.25	11.7	12.5

[1] Level required for significance: for treatments at .05 = 1.5, at .01 = 2.2
for depths at .01 = 1.1

[2] Level required for significance: for treatments at .05 = 1.5, at .01 = 2.1
for depths at .05 = 0.6, at .01 = 0.9

A summary of soil temperature data measured with mercurial ther mometers is presented in Table 3. At both the 1 and 3 inch depths th soil temperatures, measured in the unmulched plots, were 8-10° F higher than in the mulched plots. Within the mulched plots the difference were relatively small.

TABLE 3

Average Soil Temperatures in °F., 1 and 3 Inch Depths, 4 p.m. Readings

Spacing and mulching treatments	August 4 - 30		September 2 - 9	
	1 " depth	3 " depth	1 " depth	3 " depth
Matted row, no mulch	82.7	81.4	80.0	78.0
3-row, corncob mulch	75.1	73.8	70.8	69.8
3-row, straw mulch	73.1	71.9	68.8	67.8
3-row, sawdust mulch	76.2	75.4	72.5	71.1
3-row, no mulch	81.4	81.0	79.5	78.6

The diurnal temperature curves for a clear day with dry surface so are presented in Figures 2 and 3. The unmulched plots registered maximum temperature at 1 inch of 108°. The straw mulch gave the low est maximum temperature of 77°, while corn cobs registered 78° ar sawdust 80°. The minimum temperatures recorded were almost the sam

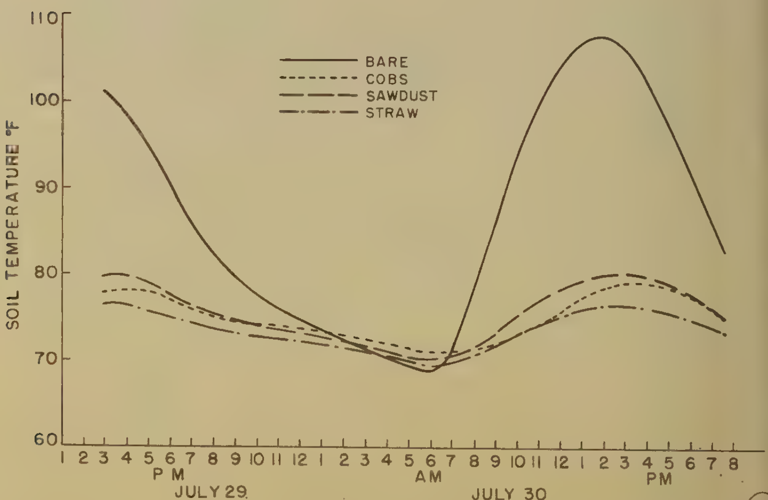

Fig. 2. Soil temperatures, 1-inch depth. Sky clear, ground surface dry

Fig.3. Soil temperatures, 3-inch depth. Sky clear, ground surface dry.

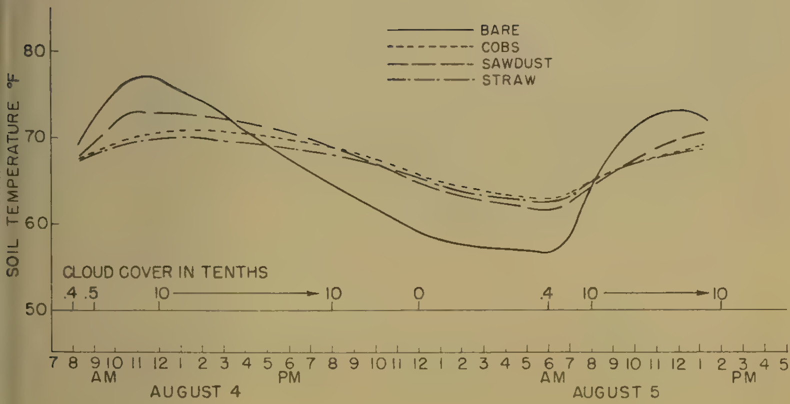

Fig.4. Soil temperatures, 1-inch depth. Soil surface wet, sky generally cloudy.

in all plots. At 3 inches the maximum temperature was 92° in the un- mulched plots. The mulched plots all registered from 76° to 78°, wit the straw plots again being the coolest.

In Figs. 4 and 5, the data for a cloudy day with moist soil are pre sented. The diurnal range of temperature was much smaller in the un mulched plots than when the sky was clear. The maximum temperatur recorded in the unmulched plots was 77°, while the mulched plots regis tered from 70°-73°.

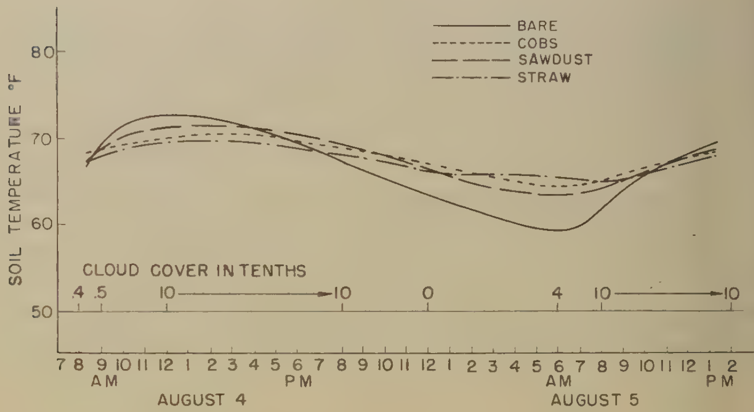

Fig. 5. Soil temperatures, 3-inch depth. Soil surface wet, sky gener- ally cloudy.

Root development observations indicated that parent plants of the mat ted row plots had considerably smaller root systems than those of th three-row plots. Comparisons among the three-row plots showed slightl deeper root systems in the unmulched plants with more new growth a greater depths. Root development was less expansive in the stra mulched plots in which runners were removed.

DISCUSSION

The most striking differences in yields of the various treatments wer between the matted row and the three-row systems. However, this can i part be accounted for by the fewer numbers of parent plants set out at th beginning of the season with the matted row. There were three times a many plants used in planting each plot in the three-row system compare to the matted row, yet the three-row system with no mulch gave mor than six times the production of berries than the unmulched matted row Undoubtedly the removal of runners exerts considerable influence o yields. The food manufactured by the parent plants of the matted ro system is channeled toward two competing factors, the production of ne

ıants and the production of berries. In the three-row systems this com-
tition is practically absent as the food is used principally in fruit pro-
ction, since runner plants are not allowed to develop. There were
me fruits produced on the new plants of the matted row towards the end
the season but they did not contribute greatly to the total yield. In
neral, the berries produced by the matted row plants were much smal-
r with fewer of marketable size.

Among the three-row treatments, the sawdust plots were the only ulching treatment which produced significantly higher yields than the mulched three-row plots. It is noteworthy that yields of the three ulched treatments were in inverse relationship to the rate of decompo-tion of the various mulching materials. This points toward the possi-lity of a nutrient tie-up in the soil from the fairly rapid decomposition organic matter in straw and to consequent lower yields. Corncobs ecompose more slowly than straw, resulting in less tie-up of nutrients nd higher yields from these plots than for the straw plots. Since sawdust ndergoes decay much slower than the other mulches, more nutrients resumably would be available to the plant and result in higher yields.

The soil temperature and moisture percentages did not vary greatly mong the mulched plots. The unmulched plots had considerably higher oil temperatures and were significantly lower in soil moisture content.

Of the unmulched plots, the three-row system had considerably less oil moisture than the matted row. This can be accounted for in that the reater number of parent plants, which were larger than the runner pro-uced plants, had a greater transpiring surface.

The bare soil of the unmulched plots would be expected to absorb a reater amount of the radiation falling on its surface than do the mulches. n addition, the heat did not have to be transmitted through a layer of oorly conducting material. The heat absorbed is used in evaporating vater from the soil and in increasing the soil temperature. The bare oil would be expected to have a greater water loss from evaporation be-cause of this larger amount of radiation absorbed, and a greater water deficiency and higher soil temperatures would result. The reduced yield of the three-row unmulched plot is probably, at least partially, due to these factors. Visual observations also indicated smaller berry size. Although the predominate effect on yield reduction in the matted row was lack of runner pulling, there was probably some effect due to higher evaporation rates from the bare soil. None of the temperatures measured were be-lieved high enough to cause any direct injury.

Comparisons in root development between the matted row and the three row systems showed the effect of runner pruning. The smaller roots of the unpruned matted row plants are in relation to the smaller parent plants and lower yields of this treatment. In like manner the larger crowns and leaf surfaces of the runner pruned plants were supported by larger root systems. The greater depth of root penetration with plants of the unmulched three-row plots compared to the mulched treatments can be explained on the basis of the soil moisture determinations. The lowest per cent of soil moisture was in the unmulched three-row system, consequently the roots penetrated to lower levels in their quest for moisture.

When one evaluates the use and effectiveness of mulches in everbearing strawberries, yield is not the only consideration. Although the unmulched

three-row plots yielded as much as the plots mulched with straw, th berries produced were definitely of inferior quality. Many of the berrie lay directly on the soil and practically all berries were splashed with so particles as a result of rains. Consequently, the berries were not mar ketable because of the grit and soil on the surface or ingrained in th fruits. This factor in itself is ample reason for the use of summe mulches on everbearing strawberries in the Midwest. Another goo reason for a mulch with the three-row system is in runner removal. runners are missed in the runner pulling operation or if the runner pull ing is necessarily delayed for a few days, there is less likelihood c runners rooting into soil under the mulch than into the unmulched soil.

Since it has been pointed out that yields from the straw plots may hav been reduced because of a nutrient tie-up as a result of decomposition c the mulch, it seems probable that an application of fertilizer befor mulching might be beneficial to yields. However, a disadvantage of stra is the likelihood of increasing the weed problem. It was noted that som oats and weed seeds, present in the straw, germinated and created th need for more hand weeding than was necessary in either the sawdust o corncob plots.

SUMMARY

Spring planted Superfection everbearing strawberries were used in a experiment to determine the effect of spacing, runner removal, and sum mer mulches on late summer yields. Soil temperature, soil moistur percentage, and root development comparisons were made between th mulching treatments. Mulching treatments were applied to plots plante according to the three-row system of spacing with continuous runner re moval and consisted of sawdust, chopped corncobs, straw, and no mulch A matted row treatment with no mulch and no runner removal was als included.

The three-row treatments with runner removal all produced signifi cantly higher yields than the matted row. Within the mulching treatment the sawdust plots gave significantly higher yields than all other treatments The straw and corncob plots did not differ significantly in yield from th unmulched three-row plots. Yields of the mulched plots were highest fo the least rapidly decomposing sawdust and lowest for the most rapidly de composing straw suggesting a tie-up of nutrients in direct relationship t the breakdown of organic matter.

Soil moisture content at 1 inch and 3 inch depths was significantly lowe in the unmulched three-row treatment than in each of the mulched treat ments. The mulched plots did not differ significantly in per cent soi moisture.

Soil temperatures, measured with mercurial thermometers at 4:0 p.m. daily at both 1 and 3 inch depths, gave readings 8 to 10° F. higher i the unmulched plots than in mulched plots. Within the mulched plots th differences were relatively small. Diurnal temperature curves, derive from the readings of a Brown recording potentiometer, are shown. Th unmulched plots registered a maximum temperature of 108° F. at 1 inc whereas the maximum for straw was 77°, for corncobs 78°, and for saw dust 80°. Minimum temperatures recorded were almost the same in al plots.

Parent plants of the matted row plots had considerably smaller root ystems than those of the three-row plots. Among the three-row plots ne unmulched plants had slightly deeper root systems with more new rowth at greater depths.

The advantages of summer mulches on everbearing strawberries are iscussed.

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IDENTIFICATION AND COLORIMETRIC DETERMINATION OF THE OXIDASES OF THE CORN ROOT TIP[1]

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There is considerable evidence that cytochrome oxidase is the princi-al terminal oxidase system in meristematic tissues of higher plants. ı corn, specifically, Maxwell (7) has reported that it is the principal xidase in young seedlings, Lundegardh (6) has reported spectroscopic vidence of the cytochrome system in the roots, and Baldovinos (1, 2) as found that the respiration of the root tip is largely cyanide - sensi-.ve indicating the predominance of a metal-containing oxidase. The ob-ect of the present work was, first, to identify and measure the oxidases f corn root tips, and, second, to adapt the colorimetric cytochrome oxi-ıase method of Smith and Stotz (10) to plant tissues.

MATERIALS AND METHODS

Seed of open pollinated WF9 X 38-7 hybrid corn was germinated on vet blotters at 30° C. until the radicles were 10 to 20 cm. long. The pical 6 mm. section was used for all enzyme preparations unless other-vise specified. The sections were blotted dry, weighed, and chopped vith a razor blade under ice-cold 0.1 M pH 7 phosphate. They were hen ground in a Potter-Elvehjem homogenizer in an ice bath with suffi-ient buffer to make a 10 percent suspension. The stock homogenate was tored at 0° C. and diluted and raised to 30° C. just before assay.

Enzyme activity was assayed at 30° C. by the colorimetric method of mith and Stotz (10) modified as follows. The reaction mixture consisted of 1 ml. of 0.0007 M leuco-dye (reduced 2,6-dichlorobenzeneoneindo-3'-hlorophenol), 0.5 ml. of 0.00021 M cytochrome c, 0.5 ml. of 0.1 M pH 7 phosphate, 0.5 ml. of enzyme suspension, and water to make 3 ml. Re-action rates were measured over an interval of approximately 15 to 60 sec. after enzyme addition. They were expressed as change in optical density per minute (=D/m) where relative values sufficed or were con-verted to microliters per hour per 0.5 ml. of 10 percent homogenate for comparison with manometric results as follows:

$$Q_{O_2} = \frac{D/m \times 60 \times 3 \times 11.2}{30.9 \times \text{dilution}}$$

[1] Journal Paper No. J-2276 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1088.

"Dilution" was the degree of dilution of the 10 percent homogenate or quivalent after centrifugation, and 30.9 was the extinction value of tl dye times the light path in cm. of the standard test tube.

Cytochrome c was prepared from beef hearts by the method of Keil and Hartree (5) and then dialyzed against water and stored frozen. Leu dye was prepared and standardized as reported previously (9) and wa treated with catalase as described in the next section.

Manometric measurements were made in air in standard Warburg ap paratus under the same conditions as in the colorimetric method excep that higher concentrations of enzyme were necessary and 0.3 ml. of 0. M hydroquinone or p-phenylenediamine was used as reductant.

RESULTS

CYTOCHROME OXIDASE METHOD

Peroxide Interference With some batches of leuco-dye initial reactio rates were higher with 1/20 or 1/40 enzyme dilutions than with the usua 1/5 dilution, but the rates decreased rapidly. This initial high rate wa caused by peroxidase action due to small amounts of H_2O_2 apparently pro duced during dye reduction. The amount of H_2O_2 in 1 ml. samples of leu co-dye was determined by measuring the difference in optical densit change in the dye system in the presence and absence of peroxidase using horse radish peroxidase or dilute corn root homogenate. In batches of leuco-dye the H_2O_2 varied from 0.020 to 0.079 micromoles pe ml. with an average of 0.055 micromoles. The use of 3 times the nor mal amount of catalyst and the bubbling of H_2 past the decolorizatio point was shown to increase the H_2O_2 content of leuco-dye more than times. It seems likely, therefore that variation in the conditions of re duction was responsible for the variation in the H_2O_2 production. It shoul also be pointed out that the use of too little catalyst and too long H_2 bub bling can lead to irreversible reduction and low leuco-dye concentration With the palladized asbestos used in the present work, 0.16 mg. per ml and 5 to 7 min. bubbling gave good results, but the best conditions fo reduction must be determined for each batch of catalyst.

The amount of catalase which would remove the H_2O_2 from the leuc dye within 1 or 2 min. also was determined with horse radish peroxidase In the routine method of 0.01 ml. of 1/100 dilution of a dialyzed commer cial catalase preparation (Sarrett) per ml. of stock leuco-dye was foun to be sufficient. In a series of 12 comparisons with different enzyme pre parations and batches of leuco-dye the catalase-treated dye gave an av erage of 18 percent higher rates as well as more reproducible and mor linear rate curves. Catalase treatment has successfully removed per oxidase interference in routine oxidase measurements due either to th spurious oxidation rate or the lowering of leuco-dye concentration.

Leuco-Dye Concentration As shown in Table 1, the concentration c leuco-dye that gave maximum rates with corn root homogenates was sim ilar to that previously reported for animal tissue homogenates (10). Wit 0.5 ml. or less of leuco-dye the reaction rates did not remain linear fo more than 20 to 30 sec. Maximum rates, which remained linear for a least 45 sec., were reached at 1.0 to 1.5 ml. though at the latter concen tration rates were more variable, possibly because of larger correction

ecessary for autoxidation. The 1.0 ml. level giving a concentration of .3 x 10^{-4} M leuco-dye was adapted for routine use.

TABLE 1

Effect of Leuco-Dye Concentration on Oxidase Activity

Leuco-Dye		Rate (D/m units)			
ml. added)	(M x 10^{-4})	7-1	7-6	8-4	8-10
0.5	1.17	0.490	0.445	0.445	0.440
1.0	2.33	0.590	0.480	0.505	0.480
1.5	3.51	0.565	0.480	0.470	0.480

Effect of Oxidized Dye Reaction rates often decreased after 60 to 90 seconds. Since the drop in leuco-dye concentration was too small to account for this decrease in rate, the possibility of inhibition by the increasing oxidized dye concentration during the reaction was investigated. This was done by measuring the effect of adding various amounts of the latter at the start of the reaction. The concentration of oxidized dye after 60 sec. at a rate of 0.600 D/m, including that caused by cytochrome c, was calculated to be 0.25 x 10^{-4} M. This concentration added at the start of the reaction caused inhibitions of 10 to 15 percent while 0.75 x 10^{-4} M oxidized dye caused 50 to 80 percent inhibition. However, it appeared that oxidized dye formed during the reaction did not have the same inhibitory effect as added oxidized dye. First, in many cases there was no significant decrease in rate during the first 60 sec. and there was never more than about 25 percent inhibition. Second, as will be shown later, reaction rates were proportional to enzyme activity even at rates up to 1.000 D/m, which would not hold if significant oxidized dye inhibition had occurred. Perhaps because of the short reaction interval used in the standard method (30 to 45 sec.), the newly formed oxidized dye did not have time to exhibit its full inhibitory effect.

Reducing Substances As reported previously (10) reducing substances in the enzyme preparation may affect the rate curves if present in sufficient amounts and if their reaction with oxidized dye occurs during the period of observation, i.e., 15 to 60 sec. after the start of the reaction. Tests on corn root homogenates with oxidized dye added to approximate the concentration in a normal oxidase reaction, however, showed reduction rates less than 0.010 D/m and no correction for reducing substances was necessary.

Cytochrome C Concentration The concentration of cytochrome c necessary for maximum rates also was found to be similar to that reported previously with animal tissue homogenates (10). As shown in Table 2, the optimum concentration was about 3.5 x 10^{-4} M. The lower rates at higher

concentrations of cytochrome c were probably due primarily to the resulting higher concentrations of oxidized dye. The unusually high rates without added cytochrome c suggested either that the cytochrome oxidase i corn root homogenate was already largely saturated with endogenous cytochrome c or that some other oxidase capable of oxidizing the leuco-dy was present. These possibilities are examined later.

TABLE 2

Effect of Cytochrome C Concentration of Oxidase Activity

Cytochrome C		Rate (D/m units)			
(ml. added)	(M X 10^{-4})	7-2	7-18	7-19A	7-19B
0.00	0.00	0.405	0.525	0.455	0.440
0.25	0.18	0.490	0.595	0.530	0.544
0.50	0.35	0.520	0.560	0.530	0.530
0.75	0.53	0.490	- -	0.445	0.495
1.00	0.70	- -	0.505	0.440	0.480

Enzyme Concentration Under the standard conditions adopted for cytochrome oxidase assay, reaction rates were found to be directly proportional to enzyme concentration over roughly a ten-fold range of concentration and up to rates of 1.000 D/m. Figure 1 shows three typical experiments. In no case did the curves go through the origin; so true blanks were determined by extrapolating to zero enzyme concentration as suggested by Slater (8). These blanks were often slightly higher than those determined without enzyme or with heated enzyme.

Enzyme Stability Tests of enzyme stability made on the undiluted 10 percent homogenate at 0°C. showed no change in activity for at least ar hour. Homogenates diluted 1/5 at 30°C. for assay were stable for about 10 minutes; consequently only 2 or 3 determinations were made on any given dilution.

IDENTIFICATION AND MEASUREMENT OF CORN ROOT TIP OXIDASES

The fact that added cytochrome c gave only a small increase in rate of leuco-dye oxidation by root tip homogenates suggested that some other oxidase might be present. This possibility was examined first by comparing the effect of added cytochrome c on supernatants and particle fractions prepared by centrifuging homogenates at 0°C. for 50 to 60 min. a 20,000 times gravity. Results of three typical experiments are summarized in Table 3. The supernatant activity was roughly half that of the whole homogenate with added cytochrome c and it showed no increase

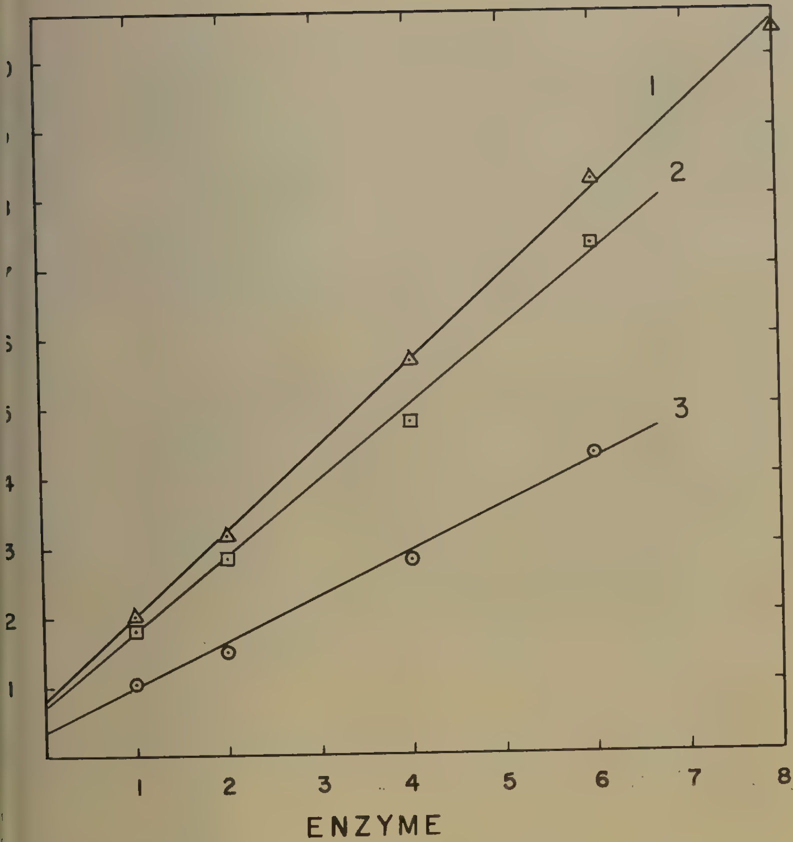

Fig.1. The effect of enzyme concentration (relative units) on rate (D/m x 10). Curves 1 and 2 are for homogenates, curve 3 for a particle fraction.

with added c. In fact, this fraction showed a small decrease, due prob ably to leuco-dye oxidation by the added cytochrome c. The supernatan activity, however, was enzymatic, since it was completely destroyed b heating at 100° C. The particle fraction, which contained the other half the oxidase activity, showed roughly a two-fold increase with added cyto chrome c and appeared, therefore, to consist largely of cytochrome ox dase. Manometric measurements on these two fractions with hydroquin one as the reductant, on the other hand, showed that almost all the activit was in the particle fraction and that the supernatant oxidase was not cap able of oxidizing hydroquinone in the presence of cytochrome c.

TABLE 3

Comparison of Oxidase Activities of Homogenate and Particle and Supernatant Fractions

Expt.	Homogenate			Supernatant			Particles		
	-C*	+C*	-C/+C	-C*	+C*	-C/+C	-C*	+C*	-C/+C
7-24	0.385	0.425	0.91	0.275	0.245	1.12	0.115	0.205	0.56
8-1	0.355	0.455	0.78	0.295	0.250	1.18	0.180	0.260	0.69
8-12	0.405	0.526	0.77	0.308	0.288	1.07	0.120	0.285	0.42

* -C: without added cytochrome c. +C: with added cytochrome c.

Both manometric and colorimetric tests were made for phenol oxi dases, the latter by the method of Smith and Stotz (9) with catechol as th substrate. In the colorimetric test no activity was found in whole homo genates. In the manometric tests catechol, hydroquinone, and p-phenyl enediamine were tried as substrates with supernatant fractions whic would be expected to contain most of the phenol oxidase. No activity wa found in one hour reaction times except for barely significant rates wit p-phenylenediamine. When the flask contents were subsequently teste colorimetrically for leuco-dye oxidation they showed definite activity indicating that lack of oxidation manometrically was not due to enzym deterioration. The possibility that the supernatant oxidase contained phenol oxidase saturated with an endogenous substrate was tested by mak ing a cold acetone precipitation of the supernatant to remove phenolic sub stances. This precipitate when tested colorimetrically with added cate chol also showed no increase in rate. The supernatant oxidase, there fore, did not appear to be a typical phenol oxidase.

The relative effect of oxidase inhibitors on the particle and superna tant fractions was next considered. No inhibition was observed with hydroxyquinoline and diethyldithiocarbamate at concentrations up to 0.017M and 0.0003M, respectively. Phenylthiourea at 0.0017M showed no inhi bition of particle oxidase and slight stimulation of supernatant oxidase further evidence against phenol oxidase in the root tips. Both oxidas fractions, however, were very sensitive to azide and cyanide, as shown Table 4. The supernatant may have been slightly more sensitive to azic than the particles but the difference was not great. On the other hand, th

upernatant was much less sensitive to cyanide than the particles. These esults are a further indication of a difference in the nature of the supertatant and particle oxidases.

TABLE 4

Effect of Cyanide and Azide on Oxidase Activity

xpt.	Enzyme Prep.	Percentage Inhibition			
		Cyanide (1.7×10^{-5} M)		Azide (1.7×10^{-4}M)	
		-C *	+C *	-C*	+C*
-1	Homogenate	--	42	--	88
	Supernatant	10	--	66	--
	Particles	80	94	47	96
3-4	Homogenate	21	37	77	78
	Supernatant	33	--	80	--
	Particles	68	91	64	87
9-21	Homogenate	52	43	--	67
	Supernatant	14	--	56	--
	Particles	68	68	57	56

* -C: without added cytochrome c: +C: with added cytochrome c

The effect of 95 percent CO-5 percent O_2 on the oxidase fractions was investigated both colorimetrically and manometrically. In the former method Thunberg tubes replaced the usual test tubes and these and the Warburg flasks were gassed by 3 successive evacuations and refills with the $CO-O_2$ and N_2-O_2 mixtures. The inhibition of homogenate activity colorimetrically ranged from 45 to 70 percent and averaged about 50 percent. Manometrically with hydroquinone as reductant, however, the inhibition was 95 percent or higher. With particles both the colorimetric and manometric method gave inhibitions of only 60 to 75 percent. Why the inhibition in this case was lower manometrically than with the homogenate is unexplained. However, the particle inhibition in the Warburg was reversed by light to the extent of 68 to 100 percent, strong evidence that this fraction was largely cytochrome oxidase. Results of CO inhibition with the supernatant in the Warburg were unsatisfactory because of low rates and relatively large heat stable blanks. Limited results with the colorimetric method indicated lower percentage inhibition than in the case of particles but the data require confirmation.

Finally, the effect of different reductants on the cytochrome oxidase of the corn root tip homogenate was investigated. Keilin and Hartree (4) and Slater (8) have shown that the relative activity of the classical heart

muscle cytochrome oxidase, with and without added cytochrome c, varied with the reductant. Similar results, summarized in Table 5, were found with the corn root tip homogenate using p-phenylenediamine and hydroquinone. The former gave much higher rates without cytochrome c than the latter, though the corresponding rates with added cytochrome c were about the same. Ratios (with or without added cytochrome c) calculated from the data of Keilin and Hartree for p-phenylenediamine and hydroquinone are 0.39 and 0.03, resp., values in good agreement with the data in Table 5. Slater has interpreted these differences among reductants to mean that some are more effective than others in reducing the endogenous cytochrome c, i.e., that attached to the oxidase particles. It appears, then, that the cytochrome oxidase of corn root tips is very much like that of heart muscle in response to reductants. It is also apparent from Table 5 that leuco-dye is more like p-phenylenediamine than hydroquinone in its ability to reduce endogenous cytochrome c.

Since the manometric method has been most widely used for cytochrome oxidase determination, it is also of interest to compare the activity determined in this way, using p-phenylenediamine and hydroquinone as reductants, with that by the colorimetric method using leuco-dye. For this comparison the particle fraction must be used in the colorimetric method to avoid the supernatant oxidase. The data in Table 5 show that the activity in corn root tips with leuco-dye is equal to or slightly higher than that with the other two reductants.

TABLE 5

Comparison of Leuco-Dye, p-Phenylenediamine, and Hydroquinone as Reductants in Oxidase Reaction

Reductant	Enzyme	No. of Determ.	-C*	+C	Diff.*	-C/+C
Leuco-Dye	Homog.	15	150 ± 6+	196 ± 7	46	—
	Particles	6	48 ± 3	86 ± 6	39	0.55
p-Phenylenediamine	Homog.	8	34 ± 4	77 ± 7	43	0.44
Hydroquinone	Homog.	5	7.4 ± 2	73 ± 8	66	0.10

* -C: without added cytochrome c; +C: with added cytochrome c; Diff.: (+C) - (-C)

\+ Rates given in microliters per hour per 0.5 ml. of 10 per cent homogenate (± S.E.)

The oxidase activities also were determined separately in the zones of cell division, enlargement, and differentiation. The root tips were divided into 2 mm. sections as described by Baldovinos (1, 2) and analyzed by the standard technic for the oxidases, by that of Smith, Robinson,

and Stotz (11) for peroxidase, and of Smith and Stotz (9) for catechol oxidase. Tests were made for catechol oxidase, even though the whole root tip had shown no activity, on the chance that sufficient enzyme would be localized in one zone to be measurable. Again, however, no activity was found. The activities of the oxidases and peroxidase in the three zones are shown in Table 6, calculated to microliters per hour per 0.5 ml. of 10 percent homogenate.

TABLE 6

Oxidative Enzyme Activity in Corn Root Tip Zones of Division, Enlargement, and Differentiation (Rates in Microliters per Hour per 0.5 ml. of 10 Per Cent Homogenate, Average of Three Experiments).

Zones	-C*	+C	Particle Oxidase+	Supernatant Oxidase+	Peroxidase
Div.	273	356	184	172	33,200
Enlarg.	140	187	104	91	13,850
Differ.	119	146	60	76	18,650

* -C: without added cytochrome c: +C: with added cytochrome c.

+ Calculated as described in text.

Separate determinations were not made on the particle and supernatant fractions but their activities can be estimated from those of the whole homogenate with and without added cytochrome c. The calculations depend on the following observations: first, the ratio of activity of particle oxidase without added cytochrome c to that with added cytochrome c equalled 0.55 (cf. Table 5); and second, the increase in rate (D) on the addition of cytochrome c is approximately the same with whole homogenate and equivalent particle fraction. Therefore, activity of particle fraction with added cytochrome c is equal to D divided by (1-0.55) and that of the supernatant fraction is the difference between the activities of whole homogenate and particle fraction.

The results indicate that both oxidase fractions and peroxidase were most abundant in the first section or division zone. In the second section or enlargement zone the oxidase activities dropped to about half and the peroxidase activity to less than half. In the third or differentiation zone the oxidase activities dropped still further while the peroxidase activity increased about a third. Finally, it is significant that in all three zones of the corn root tip the peroxidase activity was roughly 100 times that of the total oxidase.

DISCUSSION

The chief advantages of the colorimetric method for cytochrome oxidase over the conventional manometric methods are speed and sensitivity.

Analyses can be completed within 4 or 5 min. after homogenizing the ti sue and the enzymatic reaction itself lasts only a minute, which minimiz enzyme deterioration. On the basis of equivalent precision of measur ments for a manometric rate of 50 microliters per hour and a colorimetr rate of 0.200 D/m and equal reaction times and volumes, only about 1/1 as much tissue is required for the colorimetric as for the manometr method. Another possible advantage of the colorimetric method is th the same basic technic can be used not only for cytochrome oxidas phenol oxidase, and peroxidase (11), but also for dehydrogenases (12).

The colorimetric method has certain special requirements which mu be considered in its application to a particular tissue. If peroxidase present, catalase treatment of the leuco-dye may be necessary to avo interference. The concentration of both leuco-dye and oxidized dye ma influence enzyme activity; the former must be at a high enough concer tration for maximum linear rates and the latter must be kept to a min mum concentration, since there is evidence that it inhibits corn root ti oxidase. It has not yet been determined whether this effect is on the par ticle oxidase (i.e. cytochrome oxidase) or the supernatant oxidase although no similar inhibition was found on cytochrome oxidase of ra tissues. Optimum concentration of cytochrome c must also be determine since too high levels may cause a decrease in rate. Borei and Renve (3) found a similar decrease with heart muscle oxidase, using p-phenyl enediamine in the manometric method. Since the leuco-dye has an appre ciable rate of autoxidation under the conditions of the enzymatic reactio it is usually necessary to make a blank correction. A heated enzym blank was satisfactory in many cases but with some homogenates an ex trapolation blank was desirable. With these precautions, the colorimetri method was shown to give rates directly proportional to enzyme concen tration over a sufficiently wide range for assay purposes.

Several lines of evidence indicated that there are two different type of oxidase activity in corn root tips. The particle fraction showed typica cytochrome oxidase properties. With leuco-dye as reductant, it appeare to be 50 to 60 per cent saturated with endogenous cytochrome c, which i similar to heart muscle oxidase under the same conditions. Furthermore the corn root tip homogenates showed the same relative activities with p phenylenediamine and hydroquinone as reductants as the heart muscl oxidase of Keilin and Hartree (4). The particle fraction also containe virtually all of the activity measurable manometrically with hydroquinone Finally, the high sensitivity to cyanide and azide and the light reversibl CO inhibition also indicated that the particle oxidase was largely, if nc wholly, cytochrome oxidase. The identity of the supernatant oxidase i less certain. Three possibilities must be considered: first, it is a solu ble or very finely divided cytochrome oxidase saturated with endogenou cytochrome c; second, it is a phenol oxidase with attached substrate; an third, it is a new type of oxidase especially effective in oxidizing the indo phenol structure. First of all, no recognized cytochrome oxidase wit these properties has been observed. Furthermore, if such an unusua cytochrome oxidase were present in the supernatant, it should have show more activity manometrically with hydroquinone or p-phenylenediamine It seems very unlikely, therefore, that the supernatant activity is cyto chrome oxidase. There was also no evidence for a typical phenol oxidas

n the supernatant, either manometrically or colorimetrically, and there was no inhibition by phenylthiourea. These results are in agreement with those of Maxwell (7) on whole corn seedlings. The remaining possibility is that the supernatant oxidase catalyzes direct oxidation of the reduced ndophenol dye. A similar so-called "dye oxidase" was observed previously in several plant tissues by Smith and Stotz (9). The response to nhibitors indicated that the supernatant oxidase was a copper or iron enzyme, and the centrifugation data that it was probably in solution. At resent, this oxidase does not correspond in its properties with any known nzyme and must be considered a new type of oxidative enzyme. Further work is necessary to determine its natural substrate and whether it is inked directly with oxygen and is, therefore, a true oxidase.

The highest oxidase and peroxidase activity on a fresh weight basis was found in the first 2 mm. zone where cell division occurs. On the other hand, Baldovinos' respiration data (1, 2) on the same basis showed considerably higher total rate or cyanide-sensitive rate in the enlargement zone than in the division zone. This would seem to indicate that xidase activity is not the limiting factor in respiration in the division zone. In fact, the particle oxidase activity, calculated in Table 6, is high nough to account for the total respiration which Baldovinos observed in either zone, so that cytochrome oxidase could be the sole terminal oxidase n corn root tips. As in many other plant tissues the function of the very high peroxidase activity in corn root tips is obscure and puzzling.

SUMMARY

The colorimetric method for cytochrome oxidase of Smith and Stotz (10) has been adapted for the assay of plant material.

Corn root tips have been shown to contain a particle fraction which has the properties of cytochrome oxidase.

A supernatant oxidase fraction of similar level of activity does not appear to be either cytochrome oxidase or phenol oxidase.

The activities of the oxidases and peroxidases in the cell division, enlargement, and differentiation zones of corn root tips were determined.

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SOME LIMNOLOGICAL FEATURES OF CLEAR LAKE, IOWA[1]

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The Iowa Cooperative Cooperative Fisheries Research Unit has been nvestigating the fish and fish populations of Clear Lake, Iowa, since 1941 1, 2, 3, 4, 5, 6, 9, 10, 13). Although limnological conditions have been recognized as factors which affect the availability and abundance of the fish stocks, comparatively few observations have been made on the limnology of Clear Lake in the past. The present investigation analyzes certain physical and chemical characteristics of Clear Lake and enumerates members of the biota so that ecological relationships may be revealed which may enable better fishery management. These limnological investigations were made from June 21 to September 10 and from October 19 to 21, 1951.

Clear Lake is an eutrophic lake located in Cerro Gordo County, north-central Iowa. It is 4.8 miles long and has a maximum width of 2.1 miles in the eastern portion. The lake basin is shallow and gradually slopes to a maximum depth of 20 feet. The average depth is approximately 12 feet, and 25 per cent of the basin is less than 7 feet in depth (Fig. 1). Clear Lake covers an area of 3,642 acres or 5.69 square miles and has a shore development (15, pp. 93-94) of 1.58.

The basin is surrounded by irregular knob-like hills and undrained marshes which characterize the Wisconsin drift. A low, conspicuous ridge, formed by the heaving and expansion of ice, encompasses much of the lake. The watershed of Clear Lake consists of only 8,400 acres of farmland and woodland, and a large part of the water supply comes from springs and underground sources (6). No surface streams flow into Clear Lake, but an outlet on the east shore ultimately joins the Cedar River.

Approximately 42 per cent of the shore line is occupied by private residences and cottages; 29 per cent by woodland; 20 per cent by resorts, cabins, and commercial establishments; 8 per cent by roadsides, grassland and miscellany; and 1 per cent by marsh. Most of the lake's margin is a narrow, sandy beach. Boulder and rubble shores are not uncommon

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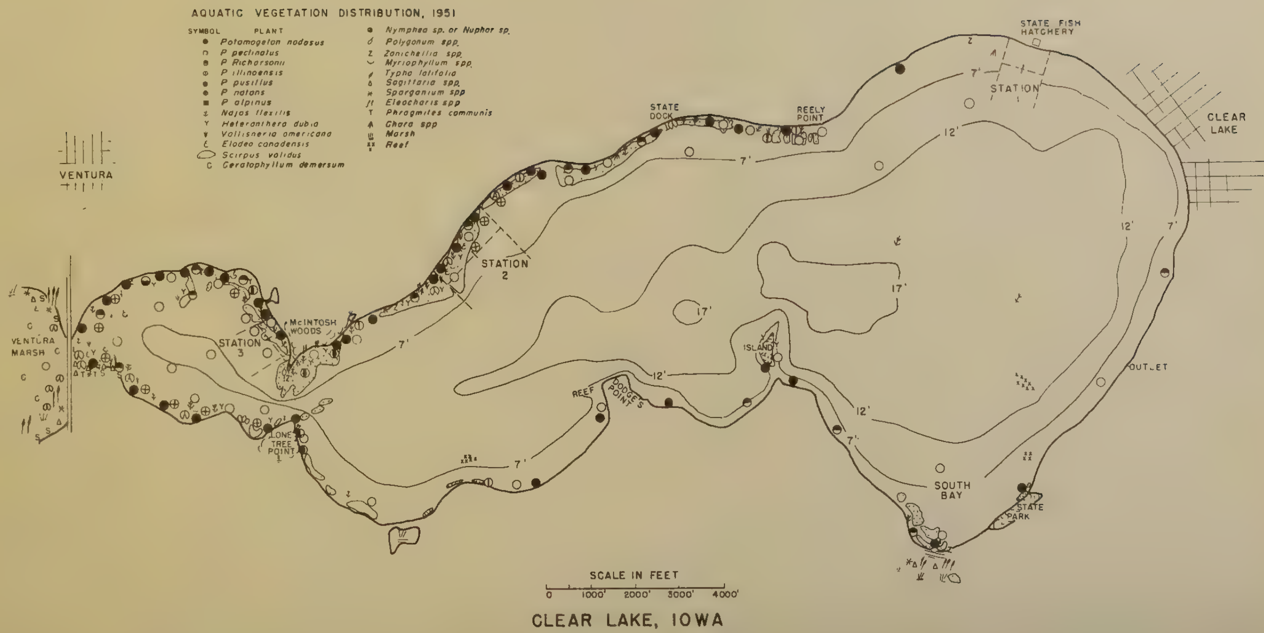

Map of Clear Lake showing depths and distribution of vegetation

n the south. Slough areas with marsh vegetation are found only in the .outhwest corner of the west end and near South Bay.

Although the area is underlain with limestone, no bedrock is exposed 1 the basin of Clear Lake. Occasional boulder reefs and rubble or gravel ottoms are present. The bottom materials of shallow water areas con- ist mainly of fine sand, which grades into a soft, organic bottom at about ne 7-foot contour in the main portion of the lake. The organic matter ontent of a deep water soil sample, as determined by the ignition method, as calculated to be 39 per cent. Other organic soils, such as pulpy peat, ibrous peat, and detritus, were found only in a few shallow, protected reas.

PHYSICAL FEATURES

Daily temperature readings were made from a thermometer submerged n 3 feet of water 200 feet off the north shore (Fig. 2). Temperature eadings were also made at different depths in the lake several times

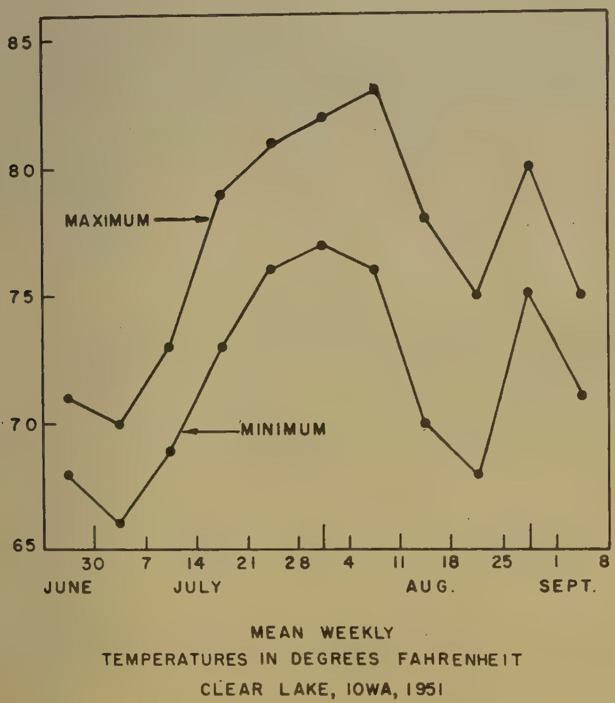

Fig. 2. Mean weekly temperatures in degrees fahrenheit, Clear Lake, Iowa, 1951.

during the summer, but only a few degrees difference was found betwee the surface and the bottom. The absence of a summer thermocline an thermal stratification is attributable to the shallow depth of Clear Lak and to wind action. A maximum summer water temperature of 86° F was reached in early August. The mean difference between daily maxi mum and minimum readings was 5.3° F.

Secchi disc readings were made throughout the summer and gave a average index of 4 feet 10 inches for light penetration. Sources of turbid ity were mainly plankton and suspended soil particles. The effect of thes sources on the secchi disc readings varied considerably during the perio of study. A general decrease in light penetration was observed as th summer progressed and was probably closely related to the increase i phytoplankton. The average reading for June was 7.3 feet; for July, 5.(feet; for August, 4.7 feet; and for September, 3.0 feet.

CHEMICAL FEATURES

The dissolved oxygen content ranged from 5 to 12.5 parts per million Supersaturation was common under favorable conditions near the surface and oxygen pulses were present in vegetated areas; the highest, 146 per- centage saturation, was over a dense bed of *Elodea canadensis*. The mear percentage saturation for dissolved oxygen in the deep water station wa 67, thus differing markedly from the average 96 and 116 per cent satura tions found at the non-vegetated and the vegetated shallow water stations respectively. It appears that even though thermal stratification wa absent, reduced circulation and less phytoplankton at greater depth resulted in lower dissolved oxygen concentrations. The average methy orange alkalinity was 156 parts per million.

PLANKTON

Macroplankton samples were collected by filtering 25 quarts or 23.65 liters of surface lake water through a No. 25 silk bolting net. Counts o the plankters were made with a Whipple ocular micrometer and a Sedwick-Rafter counting cell. In all cases ten fields (=10 cubic milli meters) were counted to give an estimate of the number of organisms pe liter of original lake water. Calculations based on the variances of the ten counts for each concentrate revealed that the following number of field would be required for various, arbitrary percentage deviations from th mean:

Percentage Deviation	Fields Required
10	134
20	35
30	16
40	9
50	6

These computations were based on twelve samples; two of the majo organisms being considered from each sample.

Bluegreen algae and diatoms were predominant, indicating a low sodium-otassium to calcium-magnesium ratio, a high conductivity, and a large mount of dissolved solids (11, 12). Although the bluegreen algae were umerically dominant, an almost equal number of species of green algae ere identified. The distribution of surface plankton organisms in Clear ake was relatively uniform, regardless of habitat. Samples from vegeated regions, however, contained more Spirogyra spp. which were ccasionally very abundant on bulrush stems and would not usually be onsidered as plankton. Crustacea were not found to be consistently more bundant in vegetated stations. It is interesting to note that in Ventura Marsh, which is choked with higher aquatic vegetation, phytoplankton was elatively scarce.

The total abundance of plankton as well as the abundance of each group f organisms fluctuated throughout the period of study, and the population resent at any one time was dependent upon pulses of certain species Table 1). A general decrease in all groups of phytoplankton in late June

TABLE 1

Abundance of Plankton Organisms per Liter, Clear Lake, Iowa

Month Av. No. Per Liter Percentage of Totals	Number of Species of Genera in Parentheses Algae Green (13)	 Algae Blue-green (15)	 Algae Diatoms (6)	 Protozoa and Rotifers (8)	 Crustacea (4)
June					
Average number	3,530	9,523	16,507	350	87
Percentage	11.77	31.75	55.03	1.16	0.29
July					
Average number	7,366	9,160	11,750	320,063	100
Percentage	2.11	2.63	3.37	91.86	0.03
August					
Average number	1,352	43,775	8,192	88,405	26
Percentage	0.95	30.88	5.78	62.37	0.02
September					
Average number	2,600	44,840	23,670	2,130	240
Percentage	3.54	61.03	32.21	2.89	0.33
October					
Average number	1,020	28,300	179,930	3,360	290
Percentage	0.48	13.29	84.51	1.58	0.14
June to October					
Average number	3,174	27,120	48,010	82,861	149
Percentage	1.97	16.81	29.76	51.37	0.09

and early July was believed to be caused by reduced transparency and light penetration following violent rains. The mean standing crop was 40,328 plankters per liter.

Green Algae

Order Tetrasporales: Gleocystis major Gerneck ex Lemmermann

Order Ulotrichales: Ulothrix sp.

Order Chlorococcales: Ankistrodesmus falcatus (Corda) Ralfs, Coelastrum microporum Naegeli, Pediastrum boryanum (Turp.) Meneghini, Pediastrum simplex (Meyen) Lemmermann, Pediastrum duplex Meyen, Scenedesmus longus Meyen, Scenedesmus quadricauda (Turp.) de Brebisson, Tetraedron limneticum Borge.

Order Zygnematales: Closterium spp. Spirogyra sp., Staurastrum spp.

Although the green algae contributed thirteen species to the plankton flora, quantitatively they were relatively unimportant and constituted only 4.1 per cent of the phytoplankton. In general, green algae were more abundant in June and early July when the lake water was still relatively cool; at that time they reached their maximum of 12,691 per liter. After mid-July the green algae were comparatively insignificant compared to other groups. Pediastrum spp. were consistently found in all samples at a concentration of about 1,315 per liter. Staurastrum spp. and Scenedesmus spp. were also common, and the remaining species appeared only occasionally.

Blue-green Algae

Order Chroococcales: Aphanocapsa elachista West and West, Aphanocapsa pulchra (Kuetz.) Rabenhorst, Aphanocapsa rivularis (Cham.) Rabenhorst, Chroococcus dispersus (Keissl.) Lemmermann, Chroococcus limneticus Lemmermann, Chroococcus turgidus (Kuetz.) Naegeli, Coelosphaerium naegelianum Unger, Coelosphaerium kuetzingianum Naegeli, Microcystis aeruginosa Kuetz., Microcystis incerta Lemmermann.

Order Hormogonales: Anabaena circinalis Rabenhorst, Anabaena flos-aquae (Lyngb.) de Brebisson, Anabaena spirodes var. crassa Lemmermann, Gleotricha echinulata (J. E. Smith) P. Richter, Lyngbya birgei G. M. Smith.

The abundance of blue-green algae was, in general, directly related to water temperature, and dense "blooms" were common in August and early September. The blue-green algae were divided into two groups on the basis of frequency of occurrence and abundance: those that were common in all samples and found consistently but never in pulse populations, and those that occur in sporadic and luxuriant "blooms" or pulses but may be completely absent in many samples. Chroococcus spp.,

phanocapsa spp., and Coelosphaerium spp. can be classified in the rst group. They were common in all samples and rarely occurred in umbers over 4,500 units per liter. Microcystis spp. are unique in that ey occurred in every sample (with an average number of 10,280 per ter) and reached a peak population of 24,080 in August. Gleotricha spp. nd Anabaena spp. on the other hand did not occur in many samples, but hen present they were usually very abundant, and, when conditions were vorable, they reached a maximum of 743,900 per liter, making the lake ater murky. At times these pulses discouraged swimming, but their ffects were only temporary, and no serious problems resulted. Blue-reen algae averaged 34.6 per cent of all the phytoplankton.

iatoms

The diatoms predominated over the blue-green algae in early summer, eclined during the warmer months, and then ascended to dominance in eptember and October when 177,930 were estimated per liter. It appears hat the diatom population was inversely related to water temperature and onsequently to the abundance of blue-green algae. Diatoms composed rom 3.4 to 84.5 per cent of the monthly plankton sample means and veraged 61.3 per cent of the entire phytoplankton population. Asterionela spp. were the predominant diatoms and were largely responsible for he fluctuations of this group. Melosira spp. were also abundant and chieved a maximum of 22,490 units in October. The other diatoms, tephanodiscus spp., Navicula spp., Surirella spp., and Fragilaria spp., vere common, though not abundant, and appeared in the majority of samples.

Protozoa and Rotifers

Although not abundant in all samples, this group comprised an average of 51.37 per cent of the entire plankton in samples analyzed. This high percentage is due to one genus, Dinobryon, which reached counts as high as 9,237,700 per liter in some samples. However, the two species, D. sertularia Ehrenberg and D. tabellariae (Lemm.) Pascher, were small and volumetrically unimportant. The maximum of this group was reached in the period before August 1, when blue-green algae, diatoms, and most other groups were relatively scarce. Ceratium hirundinella (O. F. Muell.) Dujardin and Keratella sp. were common in most samples, whereas Volvox globator L., Vorticella sp., Brachionus spp., and Trichocera sp. were found only occasionally in samples.

Crustacea

Daphnia sp., Bosmina sp., Diaptomus sp., and Cyclops sp., were observed usually in low numbers, averaging about 100 units per liter and comprising 0.09 per cent of the total plankton.

BOTTOM FAUNA

Bottom fauna samples were taken at 4 stations (Fig.1): 3 shallow water stations on sand bottoms from 2 to 4 feet in depth, and one deep

water station in the east end in water over 7 feet in depth where muck wa the predominant bottom material. Station 1 was near the fish hatchery i a non-vegetated area. Station 2 was similar except for the presence c vegetation, and Station 3, in the west end of the lake, was also vegetated

An analysis of variance indicated that the mean differences among th three shallow water stations were not statistically significant at the per cent probability level on the basis of volume (F=0.5237) or on th basis of numbers (F=0.7852) (Table 2). In general, a greater number o species were taken from the vegetated stations than from the non-vegetatec station. During August and September representatives of the bottom faun were noticeably less abundant in the non-vegetated station (No. 1) than i the other two stations.

TABLE 2

Milliliters of Bottom Fauna per Square Foot, Shallow Water Stations, Clear Lake, Iowa, 1951, and an Analysis of Variance

Station 1				Station 2				Station 3		
July	Aug.	Sept.	Oct.	July	Aug.	Sept.	Oct.	Aug.	Sept.	Oct.
1.05	0.10	0.05	5.60	0.25	3.05	0.35	3.50	0.95	1.40	3.60
	0.10	0.05	6.00	1.55	0.45	0.15	1.70	1.90	0.05	1.60
	0.30	0.10	1.10	0.65	0.10	1.60	4.50	0.65		1.50
	0.15	0.10	0.60		0.05	0.15		0.20		3.60
	0.25	0.05	2.50		0.30	0.15		0.95		0.50
	0.45		1.80			0.25				
						0.40				
						0.40				
						0.15				
Mean:										
1.05	0.25	0.05	2.95	0.80	0.80	0.40	3.25	0.95	0.75	2.15

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Among stations	2	1.3461	0.6731	0.523
Months within stations	8	53.4737	6.6842	5.201
Samples within months	39	50.1217	1.2852	
Total	49	104.9415	2.1417	

Scuds (Hyalella knickerbockeri) and tendipedid or midge larvae were the most abundant shallow water organisms. Hyalella comprised an av erage of 53.7 per cent of the volume and 52.9 per cent of the numbers o invertebrates from all shallow water stations combined. Tendipedid were usually second in abundance but were the dominant taxonomic grou at Station 1 where vegetation was absent. Midge larvae composed a average of 32.1 per cent by volume and 33.2 per cent by numbers of th

ottom fauna at the shallow water stations. Quantitatively other organisms were relatively unimportant.

The average volumes of organisms and the average total numbers of bottom organisms, taken in different months, were found to be significantly different at the 5 per cent level (volume, F=5.2010; numbers, F=.6237). There was a decline of bottom forms to an average low for all hallow water stations of 0.35 ml. per square foot in September. The aximum volume of bottom fauna per square foot was found in October, hen the mean volume for all shallow water stations was 2.70 ml.

At the deeper water station, the average volume of bottom fauna was .90 ml. per square foot. Tendipedid larvae, predominantly _Tendipes entans_, were dominant, comprising 94.5 per cent of the fauna by volume nd 55.5 per cent by numbers. _Planaria_ spp. were common and comrised 20.6 per cent of the numbers of organisms in deep water. The uly samples were largest, and with each consecutive month the volume er square foot decreased (Table 3), but the differences were not statisically significant at the 5 per cent confidence level (F=1.517 on the basis f numbers and F=1.748 on the basis of volume; an F value of over 3.59 would be significant at the 5 per cent level).

TABLE 3

Milliliters of Bottom Fauna per Square Foot, Deep Water Station, Clear Lake, Iowa, 1951

	July	August	September	October
	7.05	4.05	2.65	3.40
	2.80	3.15	1.60	1.20
	2.20	3.80	2.45	0.50
Mean	4.02	3.67	2.23	1.70

F (among months)- 1.517

The following invertebrates were collected from Clear Lake during the period of study. Although most of the invertebrates were collected from the benthos, insects were also taken from the periphyton and neuston.

Turbellaria

Planaria sp. These free living flatworms were present at all stations and very abundant in some shallow water samples, where sand bottom predominated, and in some deep water samples.

Nematoda

Free living nematodes were found only rarely in bottom samples from deep water and were insignificant in numbers and volume.

Oligochaeta

Naididae: *Stylaria lacustris* Linnaeus. These small, aquatic oligo chaetes were found only at Station 2 and 3 in October.

Tubificidae: Tubificids were common in bottom samples from shallo and deep water. They were consistently most abundant at Station throughout the period of study.

Hirudinea

The distribution of leeches was very uneven--most samples containe none, but samples from each station had considerable numbers. Sinc their size was also subject to extreme variation, they frequently distorte the quantitative determinations and were excluded in the volumetric anal ysis.

Gastropoda

Snails were collected in bottom samples from all stations. They wer always most abundant at Station 2 on aquatic plants, and rarest in dee water samples.

Physa sp. This was the common snail of Clear Lake in 1951.

Helisoma sp. Also common but not as abundant as *Physa*.

Lymnaea sp. Rare. Only one specimen being collected on aquati plants.

Pelecypoda

Sphaeriidae: These small bivalves were not common and were col- lected at Station 2, where they were most abundant, and at Station 1 *Piscidium* sp. and *Musculium* sp. were identified.

Amphipoda

Hyalella knickerbockeri (Bate). Scuds were very abundant in Clea Lake and were believed to be associated with algae and higher vegetation Although not a member of the benthic fauna, they were often taken in bot- tom samples, especially when vegetation was present. Scuds were impor tant food for many young fish. They were common and often very abundan in samples from vegetated stations, less abundant at Station 1, and rar in deep water samples. The maximum number of scuds for all station was in October.

Isopoda

Asellus communis Say. Rare. This aquatic isopod was collected o the rock bottom near the island and in one bottom sample from Station 2

Ostracoda

Many ostracods were noted while picking fresh samples, but they wer difficult to find in preserved samples. These organisms were not con sidered quantitatively due to their small size.

Hydrachnidae

A few water mites were collected at all stations during the study perio

ıd, although their fish food value is questionable, they are included in e quantitative analysis since they do not distort the volumetric deter- ıinations.

nisoptera (dragonflies)

Anax junius (Drury). This dragonfly nymph was present throughout ıe summer months and was the only species collected from Clear Lake. nax junius was generally found in dense vegetation and was not taken in ottom samples.

ygoptera (damselflies)

Damselfly nymphs were common in bottom samples at Station 2, rare t other shallow water stations, and absent in deep water. Collecting ıdicated a preference for a dense, weedy habitat. The following imma- ıres were collected from Clear Lake: Enallagma civile (Hagen), E. ageni Walsh, Enallagma spp., and Ischnura sp.

phemeroptera (mayflies)

Caenis sp. was the only mayfly nymph taken in bottom samples. It was abundant and present at all stations, except deep water, and preferred sandy bottom.

One specimen of Heptagenia sp. was collected; it was found clinging o a rock near the island.

Hemiptera (true bugs)

No Hemiptera or true bugs were taken in bottom samples. All the ollowing specimens were collected with a dip net in dense vegetation.

Back swimmers apparently were not abundant in Clear Lake. Imma- ture Notonecta undulata Say were collected among algae and dense vege- tation.

One adult giant water bug, Belostoma fluminea Say, was collected among the north shore vegetation.

Adult water boatmen, Sigura alternata Say, were taken only occasion- ally from vegetated areas.

Water treaders, Mesovelia mulsanti White, were common on the sur- face film above dense beds of algae and other plants where wave action was not severe.

Water striders, Gerridae, were members of the neuston in protected bays.

Trichoptera (caddis flies)

Agraylea multipunctata Curtis. Larvae of this species of caddis fly were collected from all stations. They were generally attached to or crawling on higher vegetation and were most abundant in deep water on Najas flexilis. Rare at the non-vegetated station.

Oecetis cinerascens (Hagen). The larva builds a distinctive log cabin-like case and was especially abundant at Station 3 in the sheltered west end attached to higher vegetation; it was less common at Station 2, and

absent from samples at the other stations.

Oecetis inconspicua (Walker). Common. This small species wa found in a sand case and showed no preference for vegetation, being mor abundant on the sand bottom of Station 1.

Oecetis sp. a of Ross. Not abundant; found only in a few sample from the shallow water, vegetated stations, mainly Station 3.

Mystacides longicornus (Linnaeus). This species was the dominar shallow water caddis fly larva of Clear Lake. It was generally in a sar and stick case attached to higher aquatic plants, but was also commo crawling on the sand bottom of the non-vegetated station.

Coleoptera (beetles)

Peltodytes edentulus (Leconte). Both adults and larvae were collecte and identified; they were rare in bottom samples and found only in dens vegetation.

Tropisternus lateralis (Fabricius). Only one adult water scavange beetle was collected from Clear Lake.

Hydrous sp. Larvae were collected in a few bottom samples.

Dineutes americanus Say. Two adult whirligig beetles were collecte from Clear Lake in July near aquatic vegetation.

Two species of predacious diving beetles were identified. Only tw adults of Coptotomus interrogatus (Fabricius) were collected. One adul Laccophilus maculosus Say. was taken near vegetation. A few beetl larvae, Laccophilus sp. were collected, probably L. maculosus.

Megaloptera

Sialis infumata Newman. Only one specimen of an alder fly larva wa collected. It was from Station 2.

Lepidoptera

Nymphula sp. Several of these aquatic Lepidoptera larvae were foun on the underside of floating leaves from Potamogeton nodosus at Station 3 They were not found at other stations.

Catoclysta sp. Rare. Found only in a few shallow water bottom sam ples from Station 1 and Station 2.

Diptera (flies)

Tendipedidae (Chironomidae):

Tanytarsus spp. Common, but not important quantitatively. Thes larvae were taken from all shallow water stations.

Pseudochironomus fulviventris (Johannsen). Small, greenish tendi pedid larvae which were found at all shallow water stations. These larva were especially abundant in October and contributed appreciably to th fall increase in volume of bottom fauna.

Chironomus (Endochironomus) spp. Common during the summe and very abundant in October samples. A medium sized, whitish larv that is often doubled up inside a translucent case. This subgenus wa described by Johannsen (8).

Tendipes (Limnochironomus) spp. Common on sandy bottoms of all ıallow water stations during the period of study.

Tendipes (Tendipes) tentans (Fabricius). A large "blood worm" hich was volumetrically the dominate form on the muck bottom of deep ater. This midge larva is extensively exploited as food by some Clear ake fish, and was the predominate food of the black bullhead (6), com-osing about 80 per cent of the diet.

Tendipes (Tendipes) decorus (Johannsen). This tendipedid occurred ı numerous bottom samples from shallow water stations, expecially here vegetation was present. Its consistently smaller size was used for basis of differentiating it from T. (T.) tentans.

Tendipes spp. Several specimens of this genus that could not be ositively identified were present in bottom sample material.

Harnischia (Harnischia) tenuicaudata (Malloch). Rare; found in a ew shallow water bottom samples taken in October.

Cryptochironomus digitatus (Malloch). Common from all shallow vater stations. A sand bottom was preferred.

Procladius spp. Common and sometimes abundant at both deep and hallow water stations. One species is believed to be more typical of leep water.

Coelotanypus sp. Common at all stations.

Heleidae (Ceratopogonidae):

Larvae were found in many samples from all stations from July to October. Large specimens were generally more characteristic of a muck bottom; and although common, they never constituted a large volume.

Anthomyiidae:

Limnophora sp. Rare; only four taken in one October bottom sample.

AQUATIC VEGETATION

In the past several years prominent changes have taken place in the aquatic vegetation of Clear Lake. Records of the state survey crew revealed that from 1945 to 1948 aquatic plants were very abundant in the shallow-water areas. Parsons (10) reports that, in 1945 and 1946, submerged vegetation was so abundant between McIntosh Woods and the Ventura grade that boating was nearly impossible and boat liveries in that end of the lake were "threatened with failure." Photographs and notes made by Cleary in 1947 showed dense beds of Potamogeton illinoensis along the northeast shore from Station 1 in front of the hatchery to Reely Point, an area which was devoid of vegetation in 1951. There was then a decline in abundance of submerged plants as well as the emergent bulrush, and vegetation was relatively scarce in 1948, 1949, and 1950. A distinct increase in the higher vegetation took place in 1951.

The species composition of the aquatic flora has also changed in recent years. According to Bailey and Harrison (1), Ceratophyllum demersum was abundant from 1941 to 1943, and Elodea canadensis and Wolffia columbiana were oommon. These plants were not found by Parsons in 1949; he reported Potamogeton nodosus as very common, a species not even reported by Bailey and Harrison. Elodea sp. and Ceratophyllum sp.

were found in 1951, the former being quite common whereas Ceratophyllum sp. was rare. The following species were collected by Parsons in 1949, but not found in 1951: Potamogeton praelongus, Spirodela polyrrhiza, Alisma plantago-aquatica, Sagittaria heterophylla, Polygonum punctatum, and Carex hystercina. Eighteen species were collected in 1951 that had not been reported by Parsons (10).

Wave action has a direct, mechanical effect on aquatic plants as well as an indirect effect by influencing bottom types. Contrary to the usual findings, shallow water vegetation was most abundant along the north shore where wave action was the greatest during the summer, in contrast to sparse beds along the more protected south shore (Fig. 1). The coarser bottom materials along the south shore probably account for the scarcity of vegetation in this area. It was believed that bulrushes helped to dissipate waves in northern communities. Although late June rains resulted in a 7 inch rise in the water level of Clear Lake, aquatic plants showed no adverse effects. Temperature was a factor in the seasonal changes of higher plants, and a decline in submerged vegetation began in September when the water became cooler.

Four major communities of aquatic flora were recognized in 1951: shallow water, reed-swamp, marginal, and deep water. These vegetation groups or communities were differentiated primarily on the basis of soil types and depth and represent different stages in the succession of a lake.

Shallow water vegetation

Shallow water communities included the majority of the species and composed the most noticeable and abundant group of Clear Lake's larger aquatic plants (Fig. 1). Dense vegetation was especially abundant along the north shore and in the western area. These communities were found from the beach to a depth of 7 feet. The soils in this range were predominately sand although appreciable quantities of muck and pulpy peat were present in the west end. Vegetation was scarce in the extreme eastern portion of the lake with the exception of South Bay. The southern shore had only a few scattered beds of vegetation. Both submerged plants and emergents are included in shallow water vegetation, Potamogeton spp. and Scirpus validus being the respective dominants from these groups.

Emergent vegetation

Scirpus validus var. creber Fern.: Softstem bulrush was the dominant and only important emergent species of the shallow water communities. Stands began at Reely Point and continued west along the north shore into the west end of the lake. Other scattered patches were found along the southern shores. Scirpus validus generally grew in water to 6 feet in depth. A hard sand bottom was preferred. It resisted wave action well, often providing a suitable environment for other species.

Phragmites communis Trin.: Reed grass was found in several small patches among the bulrushes in the vicinity of state dock.

Typha latifolia L.: Several small stands of cattail were observed

rong the north shore. This plant prefers shallow water and an organic bttom.

ubmerged vegetation

Potamogeton nodosus Poir.: This broad-leaf pondweed has the widest stribution among the shallow water Potamogeton spp. and was commonly ne dominant submergent. It was very abundant on the shore side of the ushes of the north shore and in the west end. Although generally found n sandy soils, it was also present on the richer, organic soils. Depths rom 1 to 3 feet of water were preferred.

Potamogeton pectinatus L.: Sago pondweed was very abundant in Clear ake and widely distributed, being found from 2 to 10 feet of water in all reas. It was noted growing on either sand or muck bottoms.

Najas flexilis (Willd.) Rostk. and Schmidt: This bushy pondweed was enerally associated with other shallow water species and often formed lense patches. All depths are inhabited from 1 to 15 feet and no preference was indicated for bottom soils.

Potamogeton richardsonii (Ar. Benn.) Rydb.: The clasping-leaf pondweed was abundant in the shallow water communities, especially in the west end. Found on sand and sometimes pulpy peat bottoms in 2 to 4 feet of water. Dense beds were common.

Chara spp.: Stonewort was especially abundant in local areas along the north shore in a foot or so of water where it often formed dense mats. Scattered plants were observed on sand bottom over most of the lake.

Elodea canadensis Michx.: This waterweed was common in shallow water vegetated areas. Very dense beds were present near the Ventura grade in the west end and occasionally along the north shore. Protected habitats with an accumulation of organic matter were preferred. This species was not reported by Parsons in 1949.

Vallisneria americana Michx.: Wild celery was common, scattered along the north shore inside the bulrushes and in the west end. Moderately rich soils were preferred in 3 feet of water.

Potamogeton illinoensis Morong: This broad-leaf pondweed was fairly common along the north shore being found on sandy bottoms in 2 to 4 feet of water.

Heteranthera dubia (Jacq.) Mac M.: Water star grass was a common species in the sandy vegetated areas of the north shore. Not listed by Parsons in 1949.

Potamogeton pusillus L.: This linear-leaved pondweed was found outside the rushes of the north shore and in the west end of Clear Lake in deeper water ranging from 4 to 7 feet. At these depths soils have

increasing amounts of muck. Not abundant. Not reported by Parsons.

Potamogeton natans L.: This floating-leaf pondweed was common among the bulrushes in the vicinity of McIntosh State Park, but absent in most other areas. Mucky-sand soils in 3 to 6 feet of water were preferred.

Myriophyllum sp.: Water milfoil was not abundant. Found mainly in the west end on sand or peaty-sand soils.

Nuphar advena (Ait.) Ait. and Nymphea tuberosa Paine: The yellow water lily was the more common although neither species was abundant. Water lilies preferred a rich bottom in 3 feet of water.

Zannichellia palustris L. The horned pondweed was found in water only several inches deep along the north shore. It formed sod-like aggregations in areas of clean sand that were subjected to wave action. Not reported by Parsons.

Polygonum coccineum Muhl. and Polygonum amphibium L. These smartweeds were found occasionally in shallow water in western areas of the lake.

Potamogeton amplifolius Tuckerm. and Potamogeton alpinus Balbis var. subellipticus (Fern.) Ogden. These broad leaf pondweeds were not common and found only rarely along the north shore among other Potamogeton spp.

Ceratophyllum demersum L. Coontail was only found in one locality at Station 2. It was very abundant in Ventura Marsh where the bottom soils are fibrous peat or pulpy peat.

Lemna minor L. This small free floating duckweed was frequently found in sheltered coves.

Cladophora fracta (Dillw.) Kuetzing. A free floating filamentous alga that was washed to the north shore in large quantities during June and early July, discouraging swimming and making seining nearly impossible. As the summer progressed and the water temperature rose, this alga disappeared. Many scuds or Hyallela sp. were associated with this plant.

Spirogyra spp. Commonly attached to rocks and often bulrushes, often found in plankton samples taken near higher vegetation.

Deep water vegetation

All vegetation which inhabited water over 7 feet deep was classified as deep water vegetation. Beyond this depth the bottom was predominately soft muck. Only two species were found in the deep water areas.

Potamogeton pectinatus L. Sago pondweed was widely distributed over

he entire lake and was found most abundant between the 7 and 12 foot contour. No dense beds were reported.

Najas flexilis (Willd.) Restk. and Schmidt. Bushy pondweed was often collected in bottom samples up to 15 feet in depth and was found in deeper water than any other flowering plant of Clear Lake.

Reed-swamp vegetation

In the southwest corner of the west end of Clear Lake and in the South Bay marsh the aquatic vegetation was in a more advanced stage of succession. The reed-swamp habitat was characterized by shallow water, an accumulation of detritus and fibrous peat, and dense populations of emergent plants (14). Arrowheads were dominant in water of about 1 foot in depth Sagittaria rigida Pursh, S. latifolia Willd., and S. latifolia Willd. forma gracilis (Pursh) Robins , whereas reed grass, Phragmites communis Trin. dominated in shallower water. Sparganium eurycarpum Engelm., bur-reed, was also abundant; spike rush or Eleocharis spp. and Polygonum spp. were common.

Marginal vegetation

Although this classification included a variety of plant types, the habitat was generally sandy shore or occasionally wet soil, and in no case was the substratum organic in nature like the reed-swamp habitat. Most of the plants were collected in the western portion of the lake near Ventura Marsh where conditions were most suitable for this type of vegetation. The following species were occasionally found on the wet soils along the shore: Echinochloa pungens, (Poir.) Rydb., Cyperus ferruginescens Boeckl., Poygonum lapathifolium L., Penthorum sedoides L., Bidens connata Muhl., and Asclepias incarnata L.

The following plants were noted on sandy beaches and bars in several inches of water around the lake: Scirpus fluviatilis (Torr.) Gray, Eleocharis spp., Salix nigra Marsh. and Salix interior Rowlee. None were abundant.

SUMMARY

1. Clear Lake is a shallow, eutrophic lake, glacial in origin, with a maximum depth of 20 feet and an area of 3, 642 acres.

2. Sand is the principal bottom material in portions of the lake less than 7 feet in depth, and muck is the dominant bottom soil in deep water beyond the 7 foot contour.

3. No thermocline was present. A maximum water temperature of 86 degrees Fahrenheit was reached in August.

4. Secchi disc readings averaged 4 feet 10 inches; plankton or suspended soil particles were the main sources of turbidity.

5. A high percentage saturation of dissolved oxygen was common in Clear Lake; free CO_2 averaged 51 parts per million and methyl orange alkalinity averaged 156 parts per million.

6. Blue-green algae and diatoms were dominant plankton organisms; blooms of Gleotricha spp. and Anabaena spp. were common, and at times Dinobryon spp. (small protozoans) were very abundant.

7. The higher aquatic vegetation was distinctly more abundant in 1951 than in the previous three years. Potamogeton spp. were the dominant shallow water submerged aquatic vegetation, whereas Scirpus validus was the dominant emergent form.

8. Statistically there was no difference in the quantity of bottom fauna between the vegetated and non-vegetated shallow water stations on the basis of numbers and volume.

9. Hyalella sp. was the dominant shallow water organism comprising 53.7 per cent of the volume; midge or tendipedid larvae comprised 32.1 per cent of the volume of the organisms from the shallow water samples.

10. A statistically significant seasonal variation in numbers and volume of bottom fauna was observed from the shallow water stations; the low of 0.35 ml. per sq. ft. was found in September and the maximum of 2.70 ml. per sq. ft. in October.

11. No seasonal variation was statistically evident in the deep water station; the dominant organism here was Tendipes tentans, a large "blood worm" that comprised 94.5 per cent of the bottom fauna and was utilized extensively for food by several species of Clear Lake fish. The mean volume of bottom fauna in deep water was 2.90 ml. per sq. ft.

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THREE YEARS' STUDY OF DDT RESIDUES ON CORN PLANTS TREATED FOR EUROPEAN CORN BORER CONTROL[1]

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DDT spray and dusts for control of the European corn borer, Pyrausta nubilalis (Hbn.), have been proved practical and economical for use on both field and canning corn. The possible contamination of corn ensilage and stover, intended for cattle feeding, by DDT residues has retarded the acceptance of this insecticide. It was therefore desirable to investigate the magnitude of these residues retained on the corn plants following recommended treatments for borer control. In addition to this very practical aspect, the nature of residues following the application of various formulations of DDT and the use of different types of application equipment were studied in the hope of finding means of improving control techniques.

In 1949, a project was set up for the study of insecticide residues on corn plants at the European Corn Borer Research Laboratory and the Insecticide Investigations Laboratory of the Bureau of Entomology and Plant Quarantine at Toledo, Ohio, and Vincennes, Indiana. The studies conducted during 1949 were limited for the most part to analyses of residues on the plants at harvest, on ensilage, and on canning-factory waste. These studies showed that appreciable residues were present on corn plants sprayed with DDT for control of the European corn borer. They further demonstrated that, if DDT was present on corn at ensiling time, it could be found in the ensilage in measurable quantities throughout the normal storage period (October to April), and that when contaminated silage was included in the rations of milch cows the DDT appeared in their milk.

In 1950, the European Corn Borer Research Laboratory was moved to Ankeny, Iowa, where study of insecticide residues on corn was continued by the Bureau of Entomology and Plant quarantine in cooperation with the

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[1]Journal paper No. J-2208 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1193.

[2]Coordinator of European corn borer research for Iowa State College and Bureau of Entomology and Plant Quarantine when these studies were conducted.

[3]Acknowledgment is made to C. H. Batcheleder and D. D. Questel, Bureau of Entomology and Plant Quarantine, for assistance given in these studies. Thanks are also due Ross Watson, Tom Walker, Dale Norris, and Don McCord for their part in gathering and preparing the samples.

Iowa Agricultural Experiment Station. Unfortunately, the cooperative experiment was not started until June of that year, and it was impossible to arrange test plots specifically designed for residue studies. Approximately 1,800 two-plant samples were analyzed for insecticide residues during 1950, most samples being taken from five- or six-replicate plots.

The cooperative study was continued in 1951. Experiments designed specifically for residue studies were set up. Approximately 5,000 samples of 10 plants each, taken from replicate plots, were analyzed.

In 1950 and 1951, a part of the cost of this investigation was paid by a grant in aid from the Geigy Company, Inc. Machinery employed in the application of dusts and sprays was loaned by Hahn, Inc., the Hagie Manufacturing Company, the Essick Manufacturing Company, and the Eckholm Manufacturing Company.

Previous Studies

The literature contains few references to insecticide residues on corn or corn plants. Decker et al. (1947) reported DDT residues on corn foliage from the application of DDT dusts, emulsions, and oil solutions on canning corn. They showed DDT residues from 14.9 p.p.m. with dusts to 117.3 p.p.m. with oil solutions. It was further demonstrated that leaves carried greater residues than stalks because of the greater ratio of surface area to weight.

Apple and Decker (1949) reported parathion residues of 6.8 p.p.m. on corn foliage 2 days after application of a 2-per cent dust, but only 0.7 p.p.m. 21 days later. Only 0.06 p.p.m. of parathion was found in silage made from this corn.

Ginsburg et al. (1950), in a study of longevity of residues on plants, reported the presence of parathion, DDT, and TDE on corn plants following five dust applications. The final dust application was made on July 1, and the samples were collected on July 12 and 13.

Anderson and Hashe (1949) found from 3 to 137 p.p.m. of DDT on husks, silks, or tips of the ears of corn treated with DDT for corn earworm control. Bacon (1950) also reported appreciable DDT residues on the husks and silks of harvested ears.

Hawkins (1951) concluded that early spray applications left less residue on corn plants than did late spray applications.

Ginsburg and Filmer (1951) report appreciable DDT residues on the husks and silks of corn plants treated with DDT for corn earwork control.

Anderson et al. (1951), in a study of DDT dust, spray, and injection treatments for control of the corn earworm, showed that significant amounts of DDT were not found on the edible portion of the ear, but husk and silk refuse of treated ears contained 46 to 208 p.p.m. of DDT.

Ginsburg et al. (1952) found no malathon residues on corn plants 21 days following a spray application.

EXPERIMENTAL METHODS

Cultural Methods

The fields of corn used for experimental spraying were either

ommercial fields leased for the experiments or fields on the Ankeny ield Station of Iowa State College. In all fields the cultural practices ollowed were those approved for commercial production of field or caning corn. No experimental varieties of corn or experimental fertilizaion or cultivation processes were employed.

pplication of Insecticides

The insecticideswere applied both from the ground and from airplanes. n the ground applications sprayers and dusters mounted on high-clearance ehicles were employed. Three of these machines are shown in Figs. 1, , and 3. Agricultural engineers of the Bureau of Plant Industry, Soils, nd Agricultural Engineering and the Iowa Agricultural Experiment Staion calibrated the machines and designed and constructed the special quipment that was added to facilitate experimental application of the inecticides.

Aerial applications were made with a Piper Cub plane equipped for oth spraying and dusting (Fig. 4).

Insofar as was practical, recommended methods (time, volume, pressure, and nozzle arrangement) of insecticide application were followed in ll experiments, although modifications were necessary in specific experiments, such as studies of timing of applications and variations of spray-volume dosage.

The rate of application of DDT was uniform throughout the experiments. Dusts were applied at the rate of 2 pounds and emulsions and suspensions at 1.5 pounds of DDT per acre. These dosages were in accord with the recommendations prepared by entomologists of the 13 North Central States (Regional Publication No. 22, Revised).

Sampling Methods

In studies of insecticide residues on plants the sampling methods are second in importance only to the actual analyses. The sampling method most applicable to a specific problem depends upon the size of the plot or area treated (volume of material available for sampling), the equipment available, and the amount of labor required for processing the samples, as well as on the degree of accuracy obtained. The wide variation in the results of analyses of 2-plant samples, obtained from the 1949 and 1950 data, indicated that the size of the sample should be increased. Therefore, plots were designed in 1951 so that eight to ten 10-plant samples could be taken from each plot without materially depleting the supply of material available for further sampling. Samples consisting of 10 plants each collected in 1951 varied in weight from 50 to 17, 667 grams.

The method of selecting corn plants in sampling was uniform through the 3 years of study. Only the center 2 or 3 rows of each plot were employed. The person taking the sample walked into the row 5 to 10 paces (to avoid turn areas and row ends) and took the nearest plant regardless of its condition. From this point he collected plants at 5- to 10-pace intervals until the sample was completed. The plants were cut 2 to 3 inches from the ground. When the entire sample had been collected, it was tied, tagged, and transported to the laboratory at Ankeny for processing.

Fig. 1. Hagie sprayer used in nozzle-arrangement and pressure-comparison experiments.

Fig. 2. Essick sprayer used in spray-volume experiments.

Fig.3. Eckholm sprayer applying an insecticide to corn plants approximately 50 inches in extended height.

Fig.4. A Piper Cub plane applying an insecticide dust to corn plants.

Methods of Preparing Samples

The method of preparing samples varied from year to year and wit the specific problem. all samples were weighed as soon as they were brought from the field, and the weight was recorded.

For Total-Residue Analysis. In 1949, the corn stalks were cut int 8-inch lengths and placed in 2.5-gallon glass jars with measured volume of benzene (1 ml. per gram). The residue was stripped from the stalk and an aliquot of the solution was forwarded for chemical analysis.

In 1950, the samples were handled in much the same manner excep that the stalks were cut to 6-inch lengths and the entire samples were placed in clean paper bags for forwarding for residue analysis.

In 1951, the number of samples and volume of corn plants handled were much greater than in previous seasons. Laboratory equipment sufficiently large to handle an entire sample was not available. For example, on one sampling date (August 15) samples with an accumulated weigh of more than 5 tons were collected. Furthermore, the cost of solven for recovery of the residue from the total sample would have been prohibitive. It was therefore necessary to devise a system of aliquoting samples.

After the samples brought to the laboratory had been weighed. th plants were cut into pieces not more than 1 inch in length. A vegetable slicer (Fig. 5) was used for this purpose until mid-July, when the corr plants were relatively soft, and a small silage chopper (Fig. 6) after that The chopped samples were collected in clean baskets or on paper. Th samples were mixed, and a 1-quart (approximately 1 pound) aliquot was weighed into a waxed-paper ice cream container. The aliquots were ther frozen and shipped to Vincennes for analysis.

Most of the frozen samples had barely thawed when they arrived at Vincennes. There was never any indication of decomposition or fermentation.

For Plant-Segment Analysis. For studies of residue distribution on different parts of the plant, it was dissected into four parts - leaf tips, leaf base, whorl, and stalk - as shown in Fig. 7. The separate parts of each sample were then weighed and packaged for shipment to the chemica laboratory for residue analysis.

Methods of Chemical Analysis

Recovery of Residue from Plants. Residues for analysis were recovered from the plants or parts of the plants by a stripping procedure. Th weighed sample was placed in a suitable glass container and a measure volume of solvent added. The container was then closed and the sampl tumbled for 1 hour in a tumbling machine described by Fahey, Cassil, and Rusk (1943) or rolled for 2 hours on the apparatus described by Batchelder and Berndt (1950). The sample was removed from the apparatus and allowed to stand for a few minutes; the solution of residue or an aliquot part was then drained into a storage bottle.

The container employed for recovery of residues was of such a size that the sample did not occupy more then two-thirds of it. The quantity of solvent used depended upon the maturity and the part of the corn plan

Fig. 5. Preparation of samples for residue analyses with a vegetable slicer and collection of an aliquot of chopped corn plants after thorough mixing of sample.

Fig. 6. Silage chopper used for cutting samples of mature corn plants.

Fig. 7. Corn plant dissected for study of residue distribution: A, stalk; B, whorl; C, leaf base; D, leaf tip.

included in the sample, and was sufficient so that it would flow over the plant surface when the sample container was rolled. With whole green plants 1 to 2 ml. per gram of sample was adequate, but dry plants required at least 5 ml. per gram. The solvent was carefully measured, and this quantity was considered the basic volume from which the aliquot for residue analysis was taken. Recovery experiments have shown that 90 to 95 per cent of the DDT added to corn samples can be recovered by this procedure. From 10 to 40 per cent of the solvent may be entrained or held up in the corn sample.

Analysis of DDT Residues. In the analysis of DDT residues benzene was employed as the solvent. The organic-chlorine method of Wichmann et al. (1946) was used in 1949, and the Stiff-Castillo (1945) method in 1950 and 1951. Fahey and Rusk (1951) have described the adaptation of these methods to analysis of DDT residues on corn and compared the accuracy and precision of the organic-chlorine method with the Schechter-Haller (1944) and Stiff-Castillo colorimetric methods of DDT analysis.

EFFECT OF SPRAY VOLUME AND PRESSURE AND NOZZLE ARRANGEMENT ON MAGNITUDE AND DISTRIBUTION OF DEPOSITS

Spray volume, spray pressure, and nozzle arrangement can be expected to affect the magnitude and distribution of insecticide deposits on

lants. There had been much speculation as to the effect of these factors n the control of the corn borer. Therefore, in 1950 and 1951, experiments were conducted to determine their effect on DDT deposits on corn. he spray treatments and methods of study were the same each year. An mulsion spray was applied at the rate of 10 gallons per acre, except in e study of spray volumes; the spray pressure employed was approximately 40 pounds per square inch except in the study of spray pressures; nd an 80-degree flat fan nozzle was used to apply all sprays except in he study of nozzles and nozzle arrangement.

In 1950, six replicate plots were set up in a field of Ohio C-92 hybrid ield corn. Two spray applications were made, on June 29 and July 10. When the first application was made, the corn plants were 36 to 40 inches n extended height. The magnitude of the deposits was determined on six -plant samples. A study of the distribution of deposit was made on suplicate 5-plant samples collected from plots employed in the comparison of nozzles and nozzle arrangement.

In 1951, two series of plots were set up, one in a field of Io-Chief canning corn, and the other in a field of Ohio C-92 field corn. The plots n the canning-corn field received four applications at 5-day intervals beginning on June 20. Samples were collected for analysis following each application. In the field-corn plots two applications were made, on June 22 and July 2. Loss of plants due to heavy rains made it necessary to reduce the samples in this experiment to 5 plants. Six 5-plant samples, one from each replicate, were collected following the final spray, and dissected to give four plant parts for residue analysis.

Spray-Volume Experiments

Inadequate spray volume may result in poor distribution of the deposit on the plant, but excessive volume may result in heavy run-off and thus reduce the magnitude of the deposit. The availability of water and the time required to fill the sprayer are economic factors that must be considered in the choice of volume of spray.

Experiments were conducted to determine the relative magnitude and distribution of DDT deposits when the spray volume was varied from approximately 2.5 to 80 gallons per acre. The sprays were applied with a high-clearance sprayer to single-row plots. Fig. 2 shows this sprayer and the arrangement of six 4-gallon tanks which made it possible to apply six treatments simultaneously. Variation in volume was accomplished by control of the nozzle orifice. The nozzles were welded to vice-grip wrenches to permit randomization of treatments by resetting of the wrenches. The nozzles employed, the volume of spray, and the quantity of DDT applied per acre are given in Table 1.

Table 2 shows the results of analysis of residues on samples collected in 1950. These data show that the average residue decreased from 32.2 p.p.m. when the spray volume was 2.5 gallons to 18.3 p.p.m. when it was 10 gallons per acre. However, there was no appreciable change in residue with further increases in spray volume.

Data obtained from the analysis of samples from the 1951 canning-corn plots (Table 3) show the mean residues on 60 corn plants after each application.

As in 1950, the minimum spray volume showed the greatest residue and there was a decrease as the spray volume was increased to 10 gallon per acre. However, the larger spray volumes gave greater residue than the 10-gallon volume.

TABLE 1

Nozzles Employed and Spray Volumes and DDT Delivered in Spray-Volume Experiments, 1950 and 1951

Planned Gallons of Spray Per Acre	Nozzle*	Actual Gallons of Spray Per Acre		Pounds of DDT Per Acre	
		1950	1951	1950	1951
2.5	800067	2.5	2.6	1.5	1.5
5.0	80015	5.1	5.3	1.5	1.6
10.0	8003	10.4	11.2	1.6	1.7
20.0	8006	20.7	20.1	1.6	1.5
40.0	8010	37.0	40.8	1.4	1.5
80.0	8020	68.4	74.1	1.3	1.4

* First two integers indicate angle of spray cone and additional integers indicate the orifice diameter.

TABLE 3

Effect of Spray Volume on DDT Deposit on Canning Corn After Each of Four Applications, 1951. Figures in Parts Per Million

Planned Gallons of Spray Per Acre* 2.5	First	Second	Third	Fourth	Average
2.5	115.6	176.0	181.6	112.0	146.3
5.0	64.6	126.7	142.5	90.4	106.1
10.0	81.9	105.2	100.8	39.8	81.9
20.0	151.1	140.0	110.3	34.8	111.5
40.0	102.9	118.4	104.3	99.3	106.2
80.0	158.4	145.3	74.9	80.6	114.8

* See table 1 for actual spray volume.

The results of the study of the magnitude and distribution of the deposits are shown in Table 4 and Fig. 8. The deposit on the whole plant was calculated as the weighted average of the deposits on the various parts.

TABLE 2

Effect of Spray Volume on DDT Residue on Corn Plants Collected on Different Dates, 1950.

Figures in Parts Per Million

Planned Gallons of Spray Per Acre*	July 14		July 18	July 24	August 1	August 8	Average
	Sample 1	Sample 2					
2.5	70.5	40.0	35.5	12.0	2.3	13.6	32.2
5.0	51.0	38.5	20.0	14.5	17.3	15.7	26.2
10.0	25.5	34.0	25.5	8.0	11.9	7.7	18.3
20.0	29.0	32.5	23.8	16.5	12.3	9.4	20.0
40.0	26.3	33.5	17.3	10.0	11.0	4.2	17.6
80.0	18.8	44.0	32.0	10.5	2.0	5.1	18.5

* See Table 1 for actual spray volume.

TABLE 4

Effect of Spray Volume on the Distribution of DDT Residues on Corn Plants, 1951

Planned Gallons of Spray Per Acre *	Plant Part	P.p.m. of DDT	Per cent of Total Residue	Per cent of Total Plant Weight
2.5	Leaf tip	402.0	68.3	15.7
	Leaf base	115.3	14.6	11.9
	Whorl	126.0	16.0	11.6
	Stalk	1.6	1.1	60.8
	Whole plant	92.7	--	--
5.0	Leaf tip	308.4	72.9	15.1
	Leaf base	127.0	20.4	10.2
	Whorl	30.7	5.4	11.3
	Stalk	1.3	1.3	63.4
	Whole plant	63.6	--	--
10.0	Leaf tip	180.0	57.5	14.2
	Leaf base	144.3	35.7	11.0
	Whorl	21.8	5.3	10.9
	Stalk	1.0	1.5	63.9
	Whole plant	44.1	--	--
20.0	Leaf tip	194.2	57.0	14.4
	Leaf base	123.3	25.9	10.3
	Whorl	54.0	12.6	11.4
	Stalk	3.4	4.5	63.9
	Whole plant	49.4	--	--
40.0	Leaf tip	149.0	44.6	13.6
	Leaf base	144.5	32.4	10.6
	Whorl	58.6	14.0	10.8
	Stalk	6.2	9.0	65.0
	Whole plant	45.4	--	--
80.0	Leaf tip	107.1	43.1	14.3
	Leaf base	79.1	23.1	10.4
	Whorl	70.1	22.6	11.4
	Stalk	6.2	11.2	63.9
	Whole plant	35.3	--	--

*See Table 1 for actual spray volume.

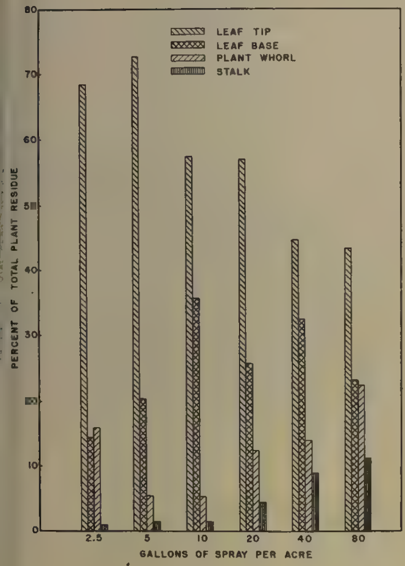


Fig. 8

Distribution of DDT residues on parts of corn plants, spray-volume studies, 1951

At all spray volumes the leaf tips show a greater deposit and a higher percentage of the total deposit than the other plant parts. When the spray volume was increased from 2.5 to 5 gallons per acre, there was a decrease in actual deposit on the leaf tips but a slight increase in the per cent of the total deposit on this part of the plant, indicating a slight excessive wetting with some run-off. Additional increases in spray volume resulted in a still greater reduction in deposit and also a reduction in the per cent of the total deposit. These data indicate that a spray volume of 2.5 to 5 gallons per acre is adequate to wet the leaf tips of the corn plants.

The leaf bases did not show as great a variation in deposit with spray volume as did the leaf tips. As the spray volume was increased from 2.5 to 40 gallons per acre, a greater quantity was deposited on the leaf bases. With a spray volume of 80 gallons per acre the leaf bases were excessively wetted and nearly half the potential deposit was carried off.

The plant whorl showed a maximum deposit when the spray volume was 2.5 gallons per acre. It is therefore assumed that this part of the plant was not thoroughly wetted and there was no run-off. Increasing the spray volume to 5 and 10 gallons per acre appreciably reduced the deposit on the whorl and the percentage of total deposit on the entire plant. The top leaves of the whorl were wetted, but the deposit was washed from these leaves to other parts of the plant. With further increases in spray volume both the deposit on the whorl and the per cent of the total deposit increased, indicating that deposit washed from the whorl leaves was lodging in the whorl cavity.

The corn stalk showed very small deposits, 1.0 and 1.6 p.p.m., when sprayed with 10 gallons or less per acre. With higher spray volumes

both the quantity and per cent of the total deposit on the stalk increase appreciably.

Spray-Pressure Studies

Spray pressure could conceivably force material down into plant crevices or even off the sprayed surface. In the studies to determine the effect of spray pressure on the magnitude and distribution of the deposits sprays were applied with the Hagie high-clearance sprayer (Fig. 1). The spray pressure of each nozzle was controlled with a pressure regulator and gauge. To maintain uniform dosage of insecticide nozzle orifices of various diameters were used.

Table 5 shows the planned pressure, the nozzle, and the actual pressure used to obtain the desired spray volume of 10 gallons per acre. The 80-degree fan nozzle was used on all plots except where a pressure of 160 pounds per square inch was used; with this treatment a 65-degree fan was found to give more nearly the planned spray pressure.

TABLE 5

Calibration of Nozzles Used in Spray-Pressure Experiments, 1950 and 1951

Planned Spray Pressure, p.s.i.	Nozzle*	Actual Spray Pressure, p.s.i.		Actual Spray volume Gallons Per Acre	
		1950	1951	1950	1951
20	8004	18	17	9.8	10.1
40	8003	33	32	9.9	10.2
80	8002	70	70	9.9	10.2
160	80015	130	--	9.9	--
160	65015	--	164	--	10.1

*First two integers indicate angle of spray cone and additional integers indicate the orifice diameter.

Table 6 shows the data obtained with different spray pressures in 1950. Although the average figures for each treatment indicate that the lowest pressure gives the greatest residue, the difference in averages (16.8 and 24.3 p.p.m.) between the maximum and minimum pressure levels employed is not great.

In the first experiment in 1951 the effect of spray pressure on the magnitude of DDT residues on canning corn was studied. The data from this experiment, given in Table 7, show that the average residue decreased slightly between spray pressures of 20 and 40 pounds per square inch, but there was little further decrease at 160 pounds pressure. Such differences would be of little importance in insect control.

In the second experiment in 1951 both the distribution and magnitude of DDT deposits at various pressures was studied. As shown in Table 8, maximum whole-plant deposit was obtained with the minimum pressure.

TABLE 6

Effect of Spray Pressure on DDT Residue on Corn Plants Collected on Different Dates, 1950.

Figures in Parts Per Million

Planned Spray Pressure, p.s.i.*	July 14		July 18	July 24	August 1	August 8	Average
	Sample 1	Sample 2					
20	46.3	46.3	26.3	12.5	10.9	6.1	24.3
40	31.0	42.5	22.5	18.5	16.5	8.3	23.2
80	38.0	42.3	15.5	18.3	8.9	7.2	21.7
160	28.0	31.0	10.8	11.8	11.7	7.7	16.8

*See Table 5 for actual spray pressures.

The distribution on the plant parts shows no appreciable difference between spray pressures.

TABLE 7

Effect of Spray Pressure on DDT Deposit on Canning Corn After Each of Four Applications, 1951. Figures in Parts Per Million

Spray Pressure, p.s.i.	First	Second	Third	Fourth	Average
20	278.1	195.6	185.1	131.7	197.6
40	269.9	189.0	157.2	111.5	181.9
80	240.0	197.5	145.3	141.5	181.1
160	237.3	201.3	178.5	109.7	181.7

Nozzle-Arrangement Studies

Many different kinds of nozzles and nozzle arrangements are used fo the application of insecticides to corn plants. The possible number o combinations of nozzles and their arrangements is limited only by th capacity of the spray machine.

For studies of the effect of nozzles and nozzle arrangements on th deposition and distribution of DDT on corn plants, the Hagie high clearance sprayer (Fig. 1) designed to spray six rows simultaneously wa used. Fig. 9 shows the nozzles used and their arrangement.

The height of the spray boom was adjusted so that the nozzle orifice would be 8 to 10 inches above the corn plants. Data relative to nozzles and orifices used are given in Table 9.

The results of analyses obtained in 1950, given in Table 10, show n practical difference in magnitude of residue between nozzle arrangements

Data from the study of the distribution of the deposit on plant parts i 1950 are shown in Table 11.

The whole plants showed the maximum deposit when three 65-degre flat fans were used side by side or two 80-degree flat fans in tandem The next greatest deposit was obtained with a single hollow-cone nozzle All the nozzle arrangements gave the heaviest deposits on leaf tips an bases, more than 90 per cent of the total on the entire plant. The tw 80-degree flat fans in tandem and the single hollow-cone nozzle gave greater deposits in the plant whorl than other nozzle arrangements.

The data from the 1951 experiment on canning corn are given in Tabl 12. The single 80-degree flat-fan and single hollow-cone nozzles gav the lowest deposits. The heaviest deposits were obtained with the two an three 65-degree fans side by side.

Data obtained in study of the distribution of deposits on corn plant from the use of different nozzle arrangements in 1951 are given i Table 13.

TABLE 8

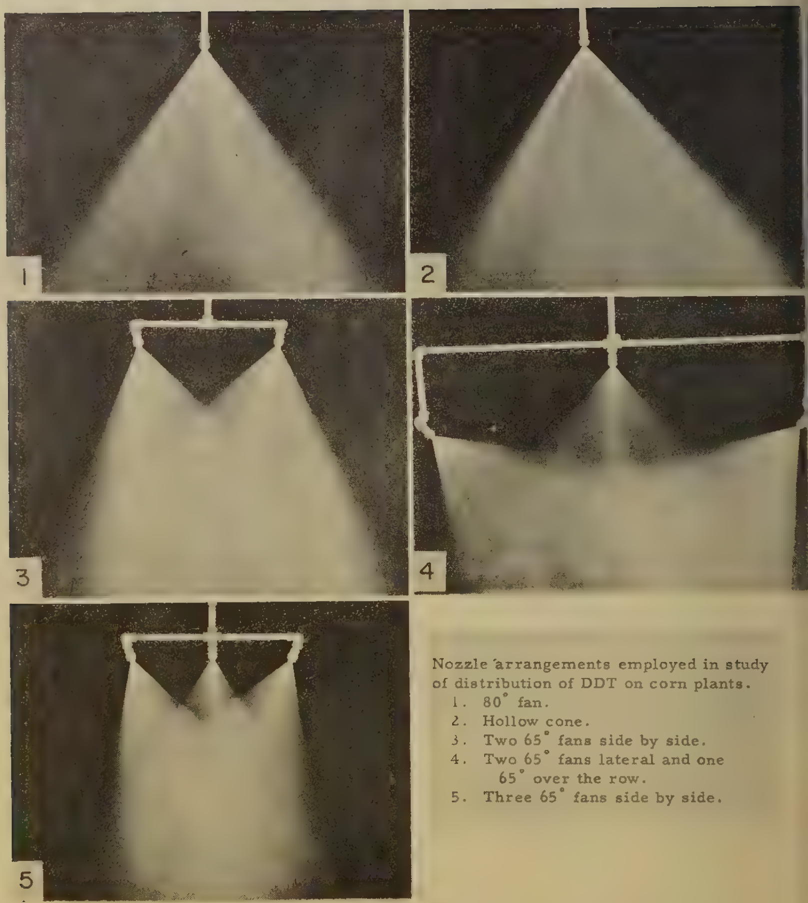
Effect of Spray Pressure on the Distribution of DDT Residues on Corn Plants, 1951

lanned Spray ressure, p.s.i.*	Plant Part	P.p.m. of DDT	Per cent of Total Residue	Per Cent of Total Plant Weight
20	Leaf Tip	493.9	67.6	11.5
	Leaf Base	155.0	28.2	15.2
	Whorl	25.1	3.3	10.9
	Stalk	1.1	0.9	62.4
	Whole Plant	83.2	--	--
40	Leaf Tip	368.1	59.9	11.2
	Leaf Base	169.2	35.6	14.3
	Whorl	22.5	3.7	11.3
	Stalk	0.8	0.8	63.2
	Whole Plant	69.1	--	--
80	Leaf Tip	341.5	67.0	12.4
	Leaf Base	137.5	27.8	12.4
	Whorl	22.9	4.0	11.3
	Stalk	1.2	1.2	63.9
	Whole Plant	62.3	--	--
160	Leaf Tip	372.7	64.4	12.0
	Leaf Base	150.0	30.6	14.1
	Whorl	23.5	4.0	11.8
	Stalk	1.1	1.0	62.1
	Whole Plant	68.8	--	--

*See Table 5 for actual spray pressure.

The single hollow cone, the single 80-degree flat fan, and two 65-legree flat fans side by side gave the lowest deposits. The maximum leposit was obtained with the three 65-degree flat fans side by side. The eaf tips and bases combined contained from 83.1 to 94.0 per cent of the otal deposit on the plant. The most important difference between nozzles was found in the plant whorl. The single 80-degree flat fan and the two 30-degree fans in tandem deposited an appreciably greater percentage of the total plant residue in the whorl than other nozzle arrangements.

The Iowa State arrangement of one 80-degree flat fan over the row with two 65-degree flat fans lateral to the row, as would be expected, resulted in lower deposits in the whorl than were obtained when all the spray was applied from flat-fan nozzles over the corn row.



Nozzle arrangements employed in study of distribution of DDT on corn plants.

1. 80° fan.
2. Hollow cone.
3. Two 65° fans side by side.
4. Two 65° fans lateral and one 65° over the row.
5. Three 65° fans side by side.

Fig. 9

TABLE 9

Arrangement and Discharge Rate of Nozzles Used for Spraying Corn Plants.

Spray Pressure 30 p.s.i. in 1950 and 34 p.s.i. in 1951.

Type and Arrangement of Nozzles	Nozzle	Number of Nozzles	Gallons of Spray Per Minute		Gallons of Spray Per Acre	
			1950	1951	1950	1951
Single Hollow Cone (1950)	D-6-23	1	0.282	--	10.5	--
(1951)	D-5-25	1	--	.282	--	10.5
80-degree Flat Fan:						
Single	8003	1	.272	.272	10.1	10.1
Two in Tandem	80015	2	.279	.269	10.3	9.9
65-degree Flat Fan:						
Two Side by Side	65015	2	.279	.279	10.3	10.3
Three Side by Side	6501	3	.272	.271	10.2	10.0
Iowa State Arrangement:						
Over Row	8001	1)				
Two Lateral to Row	6501	2)	.273	.273	10.0	10.1

TABLE 10

Effect of Nozzle Arrangement on DDT Residues on Corn Plants Collected on Different Dates, 1950. Figures in Parts Per Million

Nozzle Arrangement	July 14		July 18	July 24	August 1	August 8	Average
	Sample 1	Sample 2					
Single Hollow Cone	31.8	47.0	30.0	30.5	13.6	16.6	28.3
80-degree Flat Fan:							
Single	22.3	45.0	18.5	14.0	14.3	12.4	21.1
Two in Tandem	42.5	41.5	26.5	24.5	15.9	13.9	27.5
65-degree Flat Fan:							
Two Side by Side	36.8	47.0	17.5	18.0	22.4	6.0	24.6
Three Side by Side	50.0	47.5	25.5	15.5	13.3	6.6	26.3
Iowa State Arrangement	40.0	47.0	22.0	20.5	14.2	9.5	25.6

TABLE 11

Effect of Nozzle Arrangement on Distribution of DDT Residues on Plant Parts, 1950

ozzle rrangement	Plant Part	P.p.m. of DDT	Per Cent of Total Residue	Per Cent of Total Plant Weight
ngle Hollow Cone	Leaf Tip	408.0	63.3	16.9
	Leaf Base	182.3	31.8	18.0
	Whorl	51.3	1.9	3.8
	Stalk	4.8	3.0	61.3
	Whole Plant	106.7	--	--
)-degree Flat Fan:				
Single	Leaf Tip	362.1	69.5	16.2
	Leaf Base	130.9	29.3	18.9
	Whorl	13.8	0.6	3.5
	Stalk	0.9	0.6	61.3
	Whole Plant	84.4	--	--
wo in Tandem	Leaf Tip	449.2	55.7	14.5
	Leaf Base	242.3	37.2	18.2
	Whorl	42.9	1.6	2.7
	Stalk	11.1	6.0	64.4
	Whole Plant	117.5	--	--
5-degree Flat Fan:				
Two Side by Side	Leaf Tip	340.6	51.5	12.7
	Leaf Base	221.1	46.2	17.3
	Whorl	28.5	1.1	3.2
	Stalk	1.5	1.2	66.8
	Whole Plant	83.4	--	--
Three Side by Side	Leaf Tip	400.0	62.1	15.9
	Leaf Base	299.0	36.4	18.7
	Whorl	11.5	0.4	4.0
	Stalk	1.7	1.1	61.3
	Whole Plant	121.0	--	--
Iowa State Arrangement	Leaf Tip	299.5	63.6	16.4
	Leaf Base	153.1	34.5	17.5
	Whorl	2.8	0.2	4.5
	Stalk	2.2	1.7	61.5
	Whole Plant	77.4	--	--

TABLE 12

Comparison of DDT Deposits from Study of Spray Nozzles on Canning Corn After Each of Four Applications, 1951. Figures in Parts Per Million

Nozzle Arrangement	First	Second	Third	Fourth	Average
Single Hollow Cone:	190.8	157.8	143.6	120.7	153.2
80-degree Flat Fan:					
Single	215.5	132.7	150.4	132.3	157.7
Two in Tandem	232.7	166.9	170.4	116.9	171.7
65-degree Flat Fan:					
Two Side by Side	264.7	200.3	178.2	127.5	192.7
Three Side by Side	266.0	146.1	189.4	158.5	190.0
Iowa State Arrangement	266.4	157.2	177.8	139.4	175.2

COMPARISON OF DDT DEPOSITS FROM DUSTS, SUSPENSIONS, AND EMULSIONS

The physical characteristics of insecticide dusts, wettable powders and emulsifiable concentrates may be expected to affect the magnitude distribution, and persistence of the deposits on plants. These propertie are so interrelated that experiments designed for the study of one may b readily employed to give data on either or both of the others. Durin 1950 and 1951, a number of experiments were designed to study the rela tionship of DDT formulation and method of application to these propertie of the deposits.

Comparison of DDT Formulations and Methods of Application

The deposition and retention of DDT on canning corn following its ap plication in dusts and in suspension and emulsion sprays were studied in 1950.

The ground applications of sprays were made with an Eckholm spraye (Fig.3) equipped with three 65-degree fan-type nozzles per row when th emulsion was used and with three hollow-cone nozzles when the suspen sion was used. The aerial applications were made with a Piper Cul plane (Fig.4).

Insecticide applications were made to the experimental plots on June 27, July 6, and 11. The corn plants were 36 inches high at the time o the first application. The plots used for ground applications were si rows wide and approximately 400 feet long and were replicated four times. The aerial applications were made in two swaths (designated as A and B) each of which was eight rows wide with a six-row overlap between plots. Four randomized plots were laid out in each flight swath in such a manne as to include plants of each corn row in every replicate.

TABLE 13

Effect of Nozzle Arrangement on Distribution of DDT Residues on Plant Parts, 1951

ozzle rrangement	Plant Part	P.p.m. of DDT	Per Cent of Total Residue	Per Cent of Total Plant Weight
ingle Hollow Cone	Leaf Tip	359.5	70.8	13.3
	Leaf Base	129.2	23.2	12.1
	Whorl	29.6	4.6	10.4
	Stalk	1.5	1.4	64.2
	Whole Plant	67.4	--	--
0-degree Flat Fan:				
Single	Leaf Tip	327.7	65.8	14.3
	Leaf Base	114.5	19.3	11.9
	Whorl	98.7	14.0	9.9
	Stalk	1.1	0.9	63.9
	Whole Plant	70.9	--	--
Two in Tandem	Leaf Tip	349.0	61.9	13.7
	Leaf Base	141.2	21.2	11.6
	Whorl	116.2	15.9	10.2
	Stalk	1.1	1.0	64.5
	Whole Plant	77.7	--	--
5-degree Flat Fan:				
Two Side by Side	Leaf Tip	339.4	65.7	13.8
	Leaf Base	139.5	23.6	11.9
	Whorl	68.6	9.7	10.0
	Stalk	1.1	1.0	64.3
	Whole Plant	69.8	--	--
Three Side by Side	Leaf Tip	429.3	70.6	14.3
	Leaf Base	137.0	17.4	11.0
	Whorl	84.7	10.8	11.1
	Stalk	1.7	1.2	63.6
	Whole Plant	86.8	--	--
owa State Arrangement	Leaf Tip	370.7	63.0	13.2
	Leaf Base	164.9	26.2	12.3
	Whorl	65.0	9.1	10.9
	Stalk	2.0	1.7	63.6
	Whole Plant	77.1	--	--

Because of the large size of the plots, it was possible to take large samples for residue analysis. In the ground-treated plots four 5-plan samples were analyzed. In order to show the distribution of deposit i aerial applications, one 2-plant sample was taken from each row of th plot.

The results of analyses of residues from the sprayed and dusted plot are given in Table 14. The initial deposits (samples immediately follow ing the applications) clearly demonstrated that with ground application a emulsion deposited more DDT on the plants than either the dust or th suspension spray, and that the suspension spray deposited more DDT tha either the 5- or 10-per cent dust. It is also shown that the residues fron emulsion sprays persisted at higher concentrations than did residue from suspension sprays or dusts.

TABLE 14

Residues from DDT Sprays and Dusts Applied to Canning Corn, 1950. Figures in Parts Per Million

Formulation	On Corn Plants (1 Plot)					On Husks at Harves (2 Plots)
	July 11	July 19	July 26	July 31	August 24	August 21
			Ground Applications			
Spray:						
Emulsion	142.0	61.6	36.6	17.9	24.7	0.6
Suspension	69.9	11.3	4.0	5.3	6.1	0.8
Dust:						
10 Per Cent	58.3	6.9	--	5.1	4.8	1.2
5 Per Cent	28.4	2.8	1.7	1.9	3.4	0.8
			Aerial Applications			
Emulsion Spray	21.4	18.5	7.3	7.3	4.1	--
Dust:						
10 Per Cent	19.0	2.4	1.5	1.7	3.1	--
5 Per Cent	27.7	0.5	0.5	3.5	1.9	--

The deposits from aerial application of DDT emulsion were only abou one-seventh as great as those from ground applications. However, thei persistence on the plants was the same by both methods of application. In the aerial applications the deposits from both DDT dusts were equal to or greater than those from the emulsion.

The data from individual analyses show extremely large difference

etween rows of the swath where dust applications were made (Fig. 10). 'he emulsion made a fairly uniform deposit on each row of the swath, but he dust gave an uneven distribution, with little or no deposit on the outer ows and a very heavy deposit on the center rows of the swath.

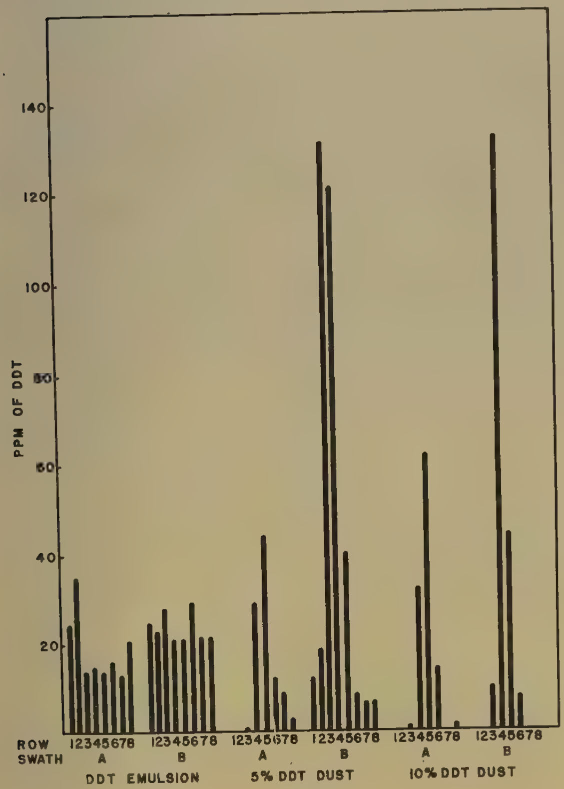


Fig. 10. Distribution of DDT residues on rows of corn of the swath from aerial application of emulsion spray and dusts. A and B are replicates of treatments.

Distribution on Plant Parts

To compare the distribution of DDT on the plant obtained with dusts emulsions, and suspensions, plots were set up in a field of Wisconsi hybrid 464 field corn in 1950. A single application was made on July 10 when the corn was approximately 35 inches in extended height.

The size of the plot made it practicable to collect duplicate 5-plan samples immediately after the application and again 3 days later. The samples were dissected and the individual parts analyzed separately.

The results of the analyses are given in Table 15. Immediately afte

TABLE 15

Comparison of DDT Residues on Various Parts of the Corn Plant Following the Application of Dusts, Emulsions, and Suspensions, 1950

Plant Part	Sampled July 10: P.p.m. of DDT	Sampled July 10: Per Cent of Total Residue	Sampled July 10: Per Cent of Total Weight	Sampled July 13: P.p.m. of DDT	Sampled July 13: Per Cent of Total Residue	Sampled July 13: Per Cent of Total Weight
			Dust			
Stalk	2.0	3.5	65.4	30.4	95.7	61.9
Whorl	16.6	2.5	5.3	0.7	0.2	5.4
Leaf Tip	160.5	57.6	13.7	0.1	3.3	16.0
Leaf Base	33.6	36.4	15.6	0.9	0.8	16.6
Whole Plant	38.0	--	--	17.4	--	--
			Suspension Spray			
Stalk	6.7	6.0	68.0	54.0	78.9	60.3
Whorl	47.2	2.1	3.4	1.6	0.2	5.3
Leaf Tip	294.2	57.5	14.5	30.3	13.5	16.8
Leaf Base	179.7	34.4	14.1	16.1	7.4	17.1
Whole Plant	85.2	--	--	38.3	--	--
			Emulsion Spray			
Stalk	4.8	3.0	61.3	5.5	10.9	65.0
Whorl	51.3	1.9	3.8	3.1	0.5	5.5
Leaf Tip	408.0	63.3	16.9	155.4	74.0	15.6
Leaf Base	182.3	31.8	18.0	33.5	14.6	13.9
Whole Plant	106.9	--	--	32.6	--	--

application the total deposits were greater from the emulsion than from the suspension, and greater from the sprays than from the dust. The distribution on the plant parts showed no appreciable differences between formulations. The leaf tips carried approximately 60 per cent of the total deposit, the leaf bases 34, the whorl about 2, and the stalk about 4 per cent.

The samples taken on July 13 showed that the residues from the dust and the suspension spray had been redistributed and that there was an appreciable loss from all three treatments. The residue on the stalks of the dusted plot had increased from 2 to 30.4 p.p.m. and from 3.5 to 95.7 per cent of the total residue, but that on the whorl and leaf tips and bases had dropped to less than 1 p.p.m. A similar but not so great a relocation of the residue is apparent on the plot sprayed with the suspension of DDT. The data from the plot sprayed with the DDT emulsion do not show this relocation. There is a small decrease in the residue on the entire plant, but the leaves still carry 83.6 per cent of the total residue, the stalks 10.9 per cent, and the whorl only 0.5 per cent. These data emphasize the adhesive property of the emulsion spray.

Comparison of Day and Night Applications

Atmospheric conditions may materially affect the deposition of insecticides. Experiments were therefore conducted in which identical treatments were applied to corn plants at night and during the day. These experiments were so designed as to permit a comparison of the magnitude of DDT residues from different formulations, their distribution on the plant parts, and their persistence.

In 1950, suspension and emulsion sprays were compared on field corn. The suspension was applied with a sprayer having three hollow-cone nozzles, and the emulsion with a sprayer having three 65-degree fan-type nozzles over each row. The first application was made on July 4, when the corn was approximately 40 inches high, and a second application on July 11. The spray plots were 6 rows wide and approximately 1,300 feet long and were replicated 4 times. The size of the plots permitted taking four samples of 5-plants from each plot.

Samples for residue analysis were collected on July 17 and at weekly intervals thereafter until September 8. A final sample was collected on October 24 at ear harvest.

Table 16 gives the average residues found at each sampling date from the two formulations applied at night and during the day, the average weight of one plant, and the average moisutre content of the plants.

In the samples taken on July 17 (6 days after the final spray) the emulsion showed only a slightly greater residue than the suspension. The night application of the emulsion deposited less DDT than the day application, but there was no difference between day and night applications of the suspension. The remaining samples taken through September 8 showed no appreciable difference between spray formulations or between day and night applications. However, samples taken October 24 showed an appreciable increase in residue over those taken September 8. This difference is attributed to the decrease in moisture content of the plants.

In 1951, the two spray formulations used in 1950 and a dust formulation

TABLE 16

Effect of Time of Application and Spray Formulation on Persistence of DDT Residues on Corn, 1950

Sampling Date	Average Weight of One Plant Grams	Per Cent Moisture	Parts per Million of DDT					
			Emulsion			Suspension		
			Day	Night	Average	Day	Night	Average
July 17	655	93.1	16.9	9.6	13.3	11.4	11.4	11.4
August 3	920	84.3	7.4	4.5	6.0	5.0	4.6	4.3
" 10-11	1067	87.0	5.1	3.1	4.1	3.9	3.3	3.6
" 15	976	82.8	3.7	2.6	3.1	3.5	2.8	3.2
" 25	1099	82.0[1]	3.2	2.4	2.8	3.0	2.9	3.0
September 1	993	81.3	2.8	2.5	2.7	3.5	2.3	3.2
" " 8	901	71.8	2.7	1.5	2.1	2.9	2.3	2.9
October 24[2]	110	3.8[3]	6.2	5.2	5.7	9.1	7.2	3.2

[1]Estimated.

[2]Corn ears not included in this sample.

ere compared in a field of 4297 hybrid corn planted on May 5. Insecti-de applications were made on June 26, when the corn was approximately 4 inches in extended height, and again on July 9. Samples for residue nalysis were collected as soon as the spray had dried and 1, 4, 8, 16, nd 48 days after spraying. The samples were dissected for separate nalysis of each part.

Table 17 shows the average residues found in plants (weighted average rom plant-parts analyses) resulting from day and night applications of usts and sprays.

TABLE 17

Residues From Day and Night Applications of DDT Dust and Suspension and Emulsion Sprays, 1951. Figures in Parts Per Million

Days After	Dust		Suspension		Emulsion	
Application	Day	Night	Day	Night	Day	Night
0	15.2	30.7	38.9	35.4	38.2	36.4
1	14.1	18.6	30.0	34.8	55.0	54.0
4	5.8	6.9	9.6	9.3	33.5	32.6
8	3.7	4.2	6.0	6.4	24.0	20.3
16	2.0	2.1	2.3	2.7	10.6	9.2
48	0.8	0.9	0.2	0.2	3.6	3.1

The night application of the dust deposited twice as much DDT as the day application. No difference is shown between day and night applications of suspension and emulsion sprays. Both sprays also show approximately the same initial deposit, but 1 day after spraying the residue from the emulsion was greater than that from the suspension.

The deposits of dust appear to persist at a higher level than those of suspension with respect to the original deposit, but the emulsion spray is the most persistent.

The distribution of DDT residue on the plant parts from the three formulations is shown in Table 18.

With the samples taken up to 16 days after application, the per cent of the total plant weight represented by the leaf tips and leaf bases is constant in all treatments. Thereafter, as the plant approached maturity, the per cent represented by the leaf tips declined sharply but the leaf bases showed little change. The percentage of the total plant in the whorl decreased during the period of study, and after 48 days the whorl was included with the plant stalk. The per cent of total plant weight represented by the stalk was greater than the total percentage of the other plant parts throughout the period of study, and increased regularly.

In the initial sample the dust showed a higher percentage of the total deposit on the leaf bases and a lower percentage on the leaf tips than was

TABLE 18

Distribution of residues on Plant Parts at Various Intervals After Treatment With DDT Dust and Sprays, 1951

Days After Application	Leaf Tip P.p.m. of DDT	Leaf Tip Per cent of Total Residue	Leaf Tip Per cent of Total Weight	Leaf Base P.p.m. of DDT	Leaf Base Per cent of Total Residue	Leaf Base Per cent of Total Weight	Whorl P.p.m. of DDT	Whorl Per cent of Total Residue	Whorl Per cent of Total Weight	Stalk P.p.m of DDT	Stalk Per cent of Total Residue	Stalk Per cent of Total Weight
Dust												
0	101.1	55.8	11.7	54.5	24.6	11.4	26.6	15.6	14.0	1.6	4.0	62.9
1	66.7	48.7	12.2	45.4	23.7	8.4	24.2	20.8	14.0	1.7	6.9	66.6
4	13.8	26.5	11.7	16.1	20.7	8.4	21.3	29.8	9.0	2.1	3.0	70.5
8	10.2	28.9	11.1	12.4	25.3	8.3	7.8	17.5	8.8	1.6	28.8	71.9
16	3.2	14.9	9.8	4.5	22.5	10.4	1.9	4.0	4.5	1.6	58.7	75.4
48	0.3	26.8	6.8	0.7	73.3	8.7	--	--	--	0	0	84.6
Suspension Spray												
0	202.6	68.8	12.3	62.9	13.9	8.3	36.0	13.5	14.0	1.7	3.8	65.4
1	184.0	67.0	11.9	68.4	16.7	8.0	35.6	12.4	11.4	1.9	4.0	68.8
4	25.4	32.9	12.3	22.5	20.8	8.9	28.0	26.9	9.1	2.7	19.5	69.9
8	18.2	33.8	11.2	17.7	23.0	8.2	11.9	16.7	8.2	2.6	27.6	72.4
16	4.1	16.3	10.2	5.4	20.8	9.6	2.0	3.0	4.0	2.0	60.0	76.3
48	1.9	55.5	6.7	1.1	44.5	9.0	--	--	--	0	0	84.4
Emulsion Spray												
0	196.5	64.4	12.3	78.1	17.8	8.7	39.3	13.6	12.9	2.2	3.8	66.2
1	309.2	67.0	11.8	120.0	17.8	8.1	54.7	11.8	11.9	2.7	3.5	68.4
4	175.2	67.3	12.8	85.0	21.1	8.4	26.5	6.7	8.4	2.3	4.9	70.5
8	132.9	61.0	9.9	78.5	28.4	8.0	9.2	3.7	8.7	2.1	7.0	73.5
16	48.9	49.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

ınd in the samples from the two sprayed plots. Initially, the whorl and :lk samples contained approximately the same percentage of the total oosit, regardless of the formulation used. In the dusted plot the leaf showed a gradual decline in per cent of total residue. The residue on leaf bases remained nearly constant, but the residues on the whorl d the plant stalk increased. The suspension spray showed a slower ss from the leaf tips, the residue on the leaf bases increased from 14 23 per cent, but the percentage on the stalk also increased through the athering periods. From the emulsion spray, the per cent of total resi- e on the leaf tips remained almost constant; there was an increase on e leaf bases, and on the whorl a decrease approximately equal to the in- ease in the total weight of the whorl. The plant stalk showed a smaller crease than did the stalks of plants dusted or sprayed with the suspension.

RELATIONSHIP OF PLANT GROWTH TO THE DEPOSITION AND RETENTION OF DDT

When an insecticide is employed to control insects on growing plants, e magnitude of the deposit and its retention at toxic levels (to the insect question) are measures of its efficiency. The presence of residues on ants may make them undesirable as food for man or animals. The mag- tude of the residue may be expected to vary with the physical properties the insecticide, the type of formulation, the rate of application, and the aracteristics of the plant material treated.

The general recommendation for the control of European corn borer 1.5 pounds of technical DDT per acre in one or two spray applications field corn, and in three or four spray applications on canning corn. he criteria for the need of sprays vary in different States and include ıch measurements as the number of corn borer egg masses per 100 ants, the amount of leaf feeding, and time-temperature specifications.

On the assumption that 1 acre of corn contains 10,000 plants, a single plication of DDT at the rate of 1.5 pounds per acre would be the equiv- ent of 68 mg. per plant. If a single plant at harvest or ensiling time eighed 1,400 grams (approximately 3 pounds) and retained all the DDT om a single spray at this dosage, the residue would amount to more an 48 p.p.m. Thus it is evident that the contamination of corn plants, tended for silage or forage, by DDT used for corn borer control is a otential hazard.

949 Experiments

In 1949, studies of DDT residues were made on fields of Silver Cross antan canning corn treated with dusts or sprays for control of second- eneration European corn borers. Three or four applications were made each field at 4- to 5-day intervals between August 6 and 19. Samples r residue analysis were collected at ear harvest, 13 to 21 days after e final application. The results, summarized in Table 19, show that e residues from sprays averaged approximately 40 p.p.m. of DDT and ose from dusts about 14 p.p.m. These data clearly demonstrate the azard of applying DDT for control of second-generation European corn orer in canning corn.

The 1949 studies were carried further to determine whether DD' present on the plants at ear harvest would contaminate silage made fron this corn, whether the DDT might be decomposed by the silage fermenta tion, and whether milk from dairy cows fed this silage would contai DDT. Corn plants from two fields that had received four spray applica tions (on August 6, 10, 15, and 19) for the control of second-generatio corn borers were used. One field was treated with a DDT emulsion an the other with a suspension. The plants in these fields contained 41. and 42.2 p.p.m. of DDT at ear harvest. The corn ears were harveste on September 1, and the corn plants were ensiled on September 3. As check a similar field of untreated corn was ensiled on another farm in th

TABLE 19

Harvest Residues From DDT Sprays and Dusts Applied On Canning Corn, 1949

Dates Applied	Formulation	P.p.m. of DDT
August 8, 12, and 17	Emulsifiable concentrate:	
	50 Per Cent	29.2
	25 Per Cent	38.4
	Colloidal, 40 Per Cent (Spray)	38.4
	Dust, 10 Per Cent	12.8
August 15, 19, and 24	Dust, 10 Per Cent	14.0
August 6, 10, 15, and 19	Emulsifiable Concentrate	
	50 Per Cent	41.0
	Wettable Powder, 50 Per Cent	42.2

same general locality. Beginning November 13, herds of dairy cow were fed silage from these two silos in their normal rations. No attemp was made to control the quantity consumed by each animal, and the silage represented only a part of the normal diet of the animals.

Silage samples for residue analysis were taken on October 10, Novem ber 15, and at approximately monthly intervals thereafter until May 16 The sample taken on October 10 was collected 7 to 8 feet below the to surface by means of a hollow tube driven into the silage. Subsequen samples were taken from the top surface or feeding level of the silo. Al silage samples were analyzed for total organic chlorine, and analyses of samples collected on January 17 and thereafter were checked by the Schechter-Haller colorimetric method.

Milk samples were collected 2 days after the first feeding of DDT contaminated silage and thereafter at monthly intervals. Each sampl

nsisted of 1-pint duplicates collected after a thorough mixing of the orning milking. All milk samples were analyzed by the Schechter-Haller ethod.

Similar analyses were made on samples of untreated silage and milk om cows fed untreated silage. The results of these experiments are ummarized in Table 20.

TABLE 20

DDT Residues Found in Silage and Milk, 1949

	Silage*		Milk
ate ollected	Level of Sample (feet)	P.p.m. of DDT	P.p.m. of DDT
ctober 10	25	22.8	--
ovember 15	22	17.6	0.6
ecember 16	18	13.6	2.0
anuary 17	12	12.4	3.8
ebruary 15	9	16.6	1.8
arch 15	6	34.2	1.2
pril 15	2	21.2	2.3
ay 16 Remaining on floor of silo (probably from previous season, 1948)		0	0.5

*Corn collected in the field on September 1, 2 to 4 days before ensiling, showed 41.6 p.p.m. of DDT.

The loss of residue in the silage, collected on October 10, may be attributed to two causes: First, water was added as the silo was filled and thus increased the weight of the silage; second, the sample of plants from the field included the corn husks, which were removed in harvesting and were therefore not present in the silage. The data available provide no explanation of the differences in the samples collected between November 15 and April 15. They could be due in part to differences in moisture content or in the distribution of leaf parts and stalk in the samples. However, at no time did the 1949 silage contain less than 10 p.p.m. of DDT, but a remnant of the silage, put in the silo in 1948, was not contaminated from the silage made in 1949.

The analyses of milk samples showed that DDT occurred in the milk soon after the initial feeding of contaminated silage and persisted for about 30 days after it was stopped.

These data show that, if DDT is present on corn plants at ensiling time, it will be found in the silage and will remain throughout the normal ensiling period. If cows are fed silage containing DDT as a part of their normal rations, DDT may be present in their milk.

1950 Experiments

Since the 1949 experiments established the fact that late-season sprays

of DDT would leave objectionable residues on corn intended for ensilage the 1950 experiments were designed to determine how late in the seasor DDT could be applied to corn plants without danger of contaminating the ensilage.

In this study a DDT suspension was applied at 20 gallons per acre Applications were made to six-row plots on June 23 and 30 and July 7 14, and 21. Three of the rows sprayed on July 7 had been sprayed or June 30, but the other three rows had not been sprayed before. This system of spraying was continued throughout the experiment. When the first spray was applied, the plants were 41 inches high and weighed 28 grams each. Samples for residue analysis were taken after each application and at intervals following the final application.

The results of these analyses (Table 21) demonstrate two very significant points: First, the smaller the corn plant at time of application, the greater was the initial deposit; second, the reduction in residue after the final application was due primarily to growth of the corn plants and weathering. Of these two factors, weathering had the greater effect with the formulation studied. When the final spray was applied on July 7 or later appreciable quantities (1 p.p.m. or more) of DDT remained on the corn plants on September 5.

TABLE 21

Parts Per Million of DDT on Corn From Suspension Sprays Applied on Various Dates, 1950

Time of Collection	Time of Application: June 23	June 30	June 30 and July 7	July 7 and 14	July 14 and 21	July 21
June 23	159.0	--	--	--	--	--
June 30	9.0	125.0	--	--	--	--
July 7	--	--	155.0	--	--	--
" 14	2.0	8.4	10.5	45.3	31.5	--
" 21	0.1	0.5	5.1	9.3	72.8	65.0
Aug. 4	0	0.1	1.0	1.9	--	--
" 18	0.3	0.3	1.3	1.4	5.6	4.9
Sept. 5	0.1	0.8	1.0	1.0	2.1	8.1

1951 Experiments

Although the 1950 experiments were made with very small samples, definite leads were developed for laying out a more comprehensive study in 1951.

Studies of the relation between spraying date and DDT residues in 1951 were conducted with two ages of corn and two DDT formulations. The plots consisted of four rows of corn 150 feet long, replicated four times for each age of corn. Corn was planted on May 4 and 16.

The first spray was applied on June 12, when the plants in the May 4 planting were 21 inches and those in the May 16 planting were 12.5 inches high. Two plots were sprayed on June 12; on June 19 one of these plots

as sprayed a second time and two additional plots were sprayed. The ame system was used in additional sprays applied on June 26 and July 5 nd 10. On July 17, one plot sprayed on July 10 and one additional plot ere sprayed. Thus in each age of corn there were six plots that reeived single applications and five plots that had been sprayed twice.

A suspension spray was applied at the rate of 20 gallons and an emulion spray at 10 gallons per acre. A four-row sprayer was used on corn hat had been planted at one time with a four-row planter.

Four samples of corn plants were collected from each plot immediately fter the spray application, after 7 and 14 days, and at ensiling time August 16). Plots that showed more than a trace of residue at ensiling ime were sampled a fifth time on October 19, at ear harvest. In plots hat received two sprays, the initial sample was taken following the last pplication.

The data obtained in these experiments are given in Table 22 and 23 nd Fig. 11 and Fig. 12.

The plants sprayed on the earlier date received heavier deposits than hose sprayed later with the same type of spray. Furthermore, small lants from the May 16 planting retained heavier residues than larger lants from the May 4 planting.

The steep slope of the curves in Fig. 11 and Fig. 12 shows the very apid loss of residue. The curves from suspension sprays are less steep han those from emulsion sprays, indicating a better retention from emulsions. This loss of residue from the plant surface is the result of both dilution by plant growth and weathering.

The effect of plant size on the initial deposit is shown in the difference n magnitude of deposits on plots receiving the fourth, fifth, or sixth application as compared with plots receiving one of the first three applications. The abrupt difference between the third and fourth applications may be due to some factor other than plant size.

With suspension sprays the effect of two applications a week apart is nearly nullified by the plant-growth dilution and weathering, but with emulsions two sprays gave appreciably greater residues than one spray.

The effect of plant size at time of application on the residues at ensiling time is clearly demonstrated. The younger the plants at time of application the more complete was the dissipation of the residue. It is again shown that the residues from emulsions persist longer than do residues from suspensions.

The results of these studies left three important questions to be answered:

1. Why were the residues from sprays applied prior to July 5 appreciably greater than those from sprays applied later?
2. What part of the residue loss between samplings can be attributed to weathering and what part to plant-growth dilution?
3. Can a definite date or unit of plant height or weight be designated as a point after which sprays will leave an appreciable residue on corn plants at ensiling or ear harvest?

Why are residues from sprays applied prior to July 5 obviously greater than the residues from the same formulation applied after July 5. It is doubtful whether this question can be satisfactorily answered. However, several theories are suggested.

TABLE 22

DDT Residues, in Parts Per Million, From Suspension Sprays on Corn, 1951

Spray No.	Date of Spraying and Sampling	From Single Applications No.1	No.2	No.3	No.4	No.5	No.6	From Two Applications Nos.1 and 2	Nos.2 and 3	Nos.3 and 4	Nos.4 and 5	Nos.5 and 6
	Corn Planted on May 4, 1951											
1	June 12	--	--	--	--	--	--	--	--	--	--	--
	" 13	158.7	--	--	--	--	--	--	--	--	--	--
2	" 19	10.1	141.8	--	--	--	--	142.3	--	--	--	--
3	" 26	3.7	11.0	99.4	--	--	--	11.4	102.6	--	--	--
4	July 5	--	1.8	3.7	43.4	--	--	3.1	4.4	66.8	--	--
5	" 10	--	--	4.7	13.6	45.4	--	--	6.8	14.3	55.9	--
6	" 17	--	--	--	9.3	12.5	46.9	--	--	12.0	13.5	56.9
	" 24	--	--	--	--	6.6	8.2	--	--	--	8.2	10.9
	" 31	--	--	--	--	--	4.1	--	--	--	--	5.5
	Aug. 16*	0	0	0	Trace	0.8	1.2	0	Trace	1.5	1.6	2.6
	Oct. 19**	--	--	--	--	0.4	0.6	--	--	1.1	4.1	2.5
	Corn Planted on May 16, 1951											
1	June 12	--	--	--	--	--	--	--	--	--	--	--
	" 13	147.1	--	--	--	--	--	--	--	--	--	--
2	" 19	6.9	135.5	--	--	--	--	139.7	--	--	--	--
3	" 26	4.7	6.2	164.3	--	--	--	10.4	156.6	--	--	--
4	July 5	--	3.8	6.7	85.3	--	--	6.0	7.3	87.6	--	--
5	" 10	--	--	5.8	15.9	66.3	--	--	5.5	14.2	76.4	--
6	" 17	--	--	--	11.3	17.9	77.6	--	--	9.5	22.7	61.4
	" 24	--	--	--	--	5.3	6.3	--	--	--	5.8	12.5
	" 31	--	--	--	--	--	4.1	--	--	--	--	3.9
	Aug. 16*	0	0	0	Trace	Trace	0.2	Trace	Trace	Trace	0.3	0.5
	Oct. 19**	--	--	--	--	0.4	Trace	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

DDT Residues, in Parts Per Million, From Emulsion Sprays on Corn, 1951

Spray No.	Date of Spraying and Sampling	From Single Applications						From Two Applications				
		No.1	No.2	No.3	No.4	No.5	No.6	Nos.1 and 2	Nos.2 and 3	Nos.3 and 4	Nos.4 and 5	Nos.5 and 6
	Corn Planted on May 4, 1951											
1	June 12	--	--	--	--	--	--	--	--	--	--	--
	" 13	219.3	--	--	--	--	--	--	--	--	--	--
2	" 19	41.9	173.0	--	--	--	--	243.3	--	--	--	--
3	" 26	15.2	22.6	144.4	--	--	--	44.2	171.1	--	--	--
4	July 5	--	4.7	8.9	75.9	--	--	8.6	16.1	78.5	--	--
5	" 10	--	--	15.9	44.6	66.6	--	--	16.2	45.4	96.0	--
6	" 17	--	--	--	30.5	28.0	72.8	--	--	23.1	39.3	93.2
	" 24	--	--	--	--	16.7	18.0	--	--	--	22.4	37.4
	" 31	--	--	--	--	--	13.1	--	--	--	--	17.6
	Aug. 16*	0	0	1.3	6.2	6.6	5.4	0	2.0	7.2	7.3	12.4
	Oct. 19**	--	--	1.4	5.7	1.3	2.3	--	1.1	4.0	5.2	5.5
	Corn Planted on May 16, 1951											
1	June 12	--	--	--	--	--	--	--	--	--	--	--
	" 13	288.4	--	--	--	--	--	--	--	--	--	--
2	" 19	60.7	233.5	--	--	--	--	254.5	--	--	--	--
3	" 26	10.0	41.2	271.3	--	--	--	30.1	287.3	--	--	--
4	July 5	--	8.1	17.5	83.5	--	--	6.7	30.5	124.9	--	--
5	" 10	--	--	14.6	38.4	120.4	--	--	16.4	73.6	163.6	--
6	" 17	--	--	--	26.1	36.9	93.9	--	--	31.7	77.2	123.3
	" 24	--	--	--	--	13.8	16.9	--	--	--	20.1	28.6
	" 31	--	--	--	--	--	13.1	--	--	--	--	17.6
	Aug. 16*	0	0	0	2.4	3.4	3.2	0	0	2.0	6.5	8.4
	Oct. 19**	--	--	--	3.4	1.1	1.0	--	--	3.0	8.0	5.6

*Corn plants matured to ensilage stage.
**Corn ears dry, suitable for ear harvest.

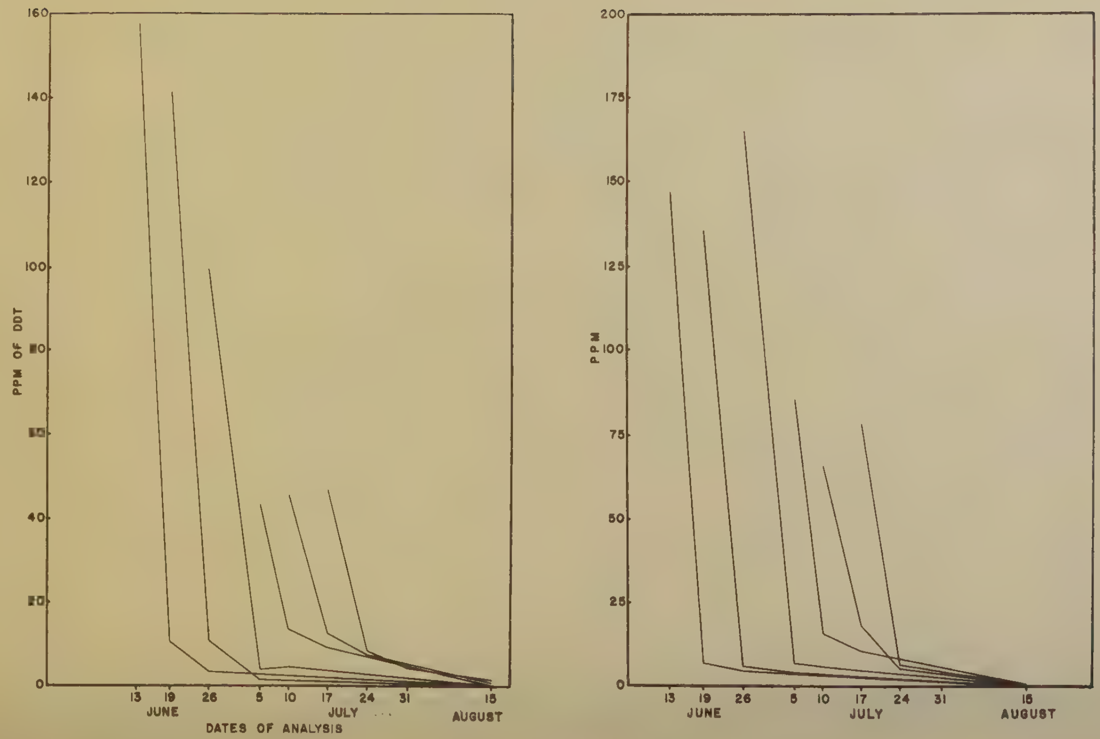


Fig. 11. Deposition and retention of DDT from suspension sprays applied on corn: Left, on corn planted on May 4, 1951; right, on corn planted on May 16, 1951.

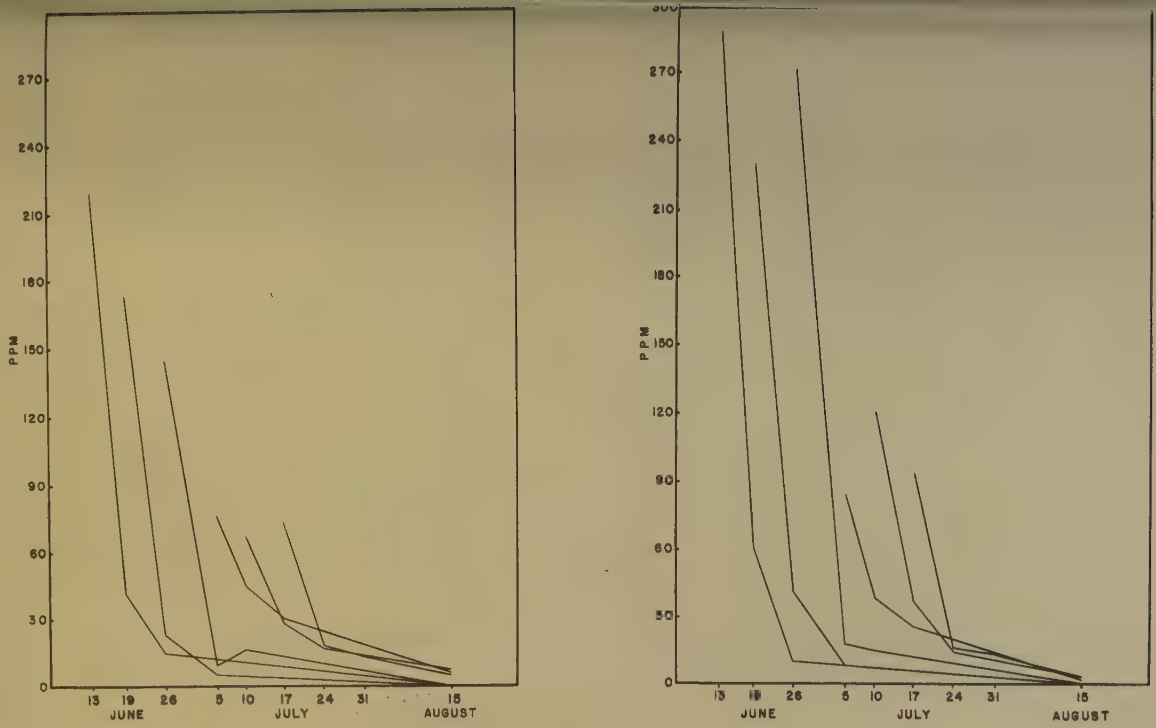


Fig. 12. Deposition and retention of DDT from emulsion sprays applied on corn: Left, on corn planted on May 4, 1951; right, on corn planted on May 16, 1951.

Fig. 13 shows the average extended height of corn plants and the average weight of one plant on various dates during the 1951 season. It will be noted that the slope of the curves depicting the average height of the plants is fairly uniform throughout the period of study. On the other hand, the curves depicting the weight of one plant are almost flat between June 13 and 19; then increases in pitch and after June 26 are steep. These curves show that early in the season (prior to June 26) the height of the plant is increasing more rapidly than its weight. The rapid increase in weight after June 26 is not accompanied by a corresponding increase in height and foliage development.

Fig. 14 shows the ratio of height to weight of corn plants on various dates during 1951. These curves for both ages of corn are very steep from June 13 to 26 and then flatten out through the remainder of the season. The ratio of height to weight affords an estimate of the ratio of plant surface to weight. When the ratio is high there is more leaf than stalk and hence, more plant surface per gram, but as the ratio decreases the relative weight of stalk increases, thus decreasing the amount of plant surface per gram of plant.

Since the DDT residues are expressed on a weight basis (parts per million), the amount of plant surface per unit of weight affects the magnitude of residues. Thus, the leafy corn plants early in the season would be expected to retain greater deposits than the heavily stocked corn plants in the latter part of the growing season.

The difference in magnitude of deposits between early (June) and late (July) applications might be due to differences in ratio of plant surface to weight and in the characteristics of the plant surface at the time of the application.

It is possible to measure the effect of both plant growth and weathering on residues. The residue in parts per million is in effect the number of micrograms per gram of plant weight; therefore, the decrease in residue with plant growth would be inversely proportional to the increase in plant weight. The effect of plant growth on residues in samples collected in 1951 is shown in Table 24. Plants weighing approximately 27 grams each when they were sprayed on June 12 weighed 1,390 grams each on August 16. The increase in weight alone would dilute the residue load approximately 98 per cent. The effect of plant growth on residues is thus a function of the increase in weight of the plants between the spray application and the final sampling.

Weathering in relation to spray residues includes the effects of precipitation, wind, and temperature. A given amount of precipitation in the form of a dew, a gentle rain, or a heavy downpour would be expected to have different effects on a residue. Similarly, a rain accompanied by wind would have a different effect than rain while the air was still. The effect of a given amount of air movement would depend upon whether the weather was dry or humid.

To study the effect of weather on residues it is necessary to eliminate the dilution effect of plant growth. This can be done by calculating the data to units of residue per plant. Table 25 and Fig. 15 show the residues per plant from single applications of suspension and emulsion sprays in 1951. The maximum and minimum temperatures and precipitation records during the period of study are given in Fig. 16.

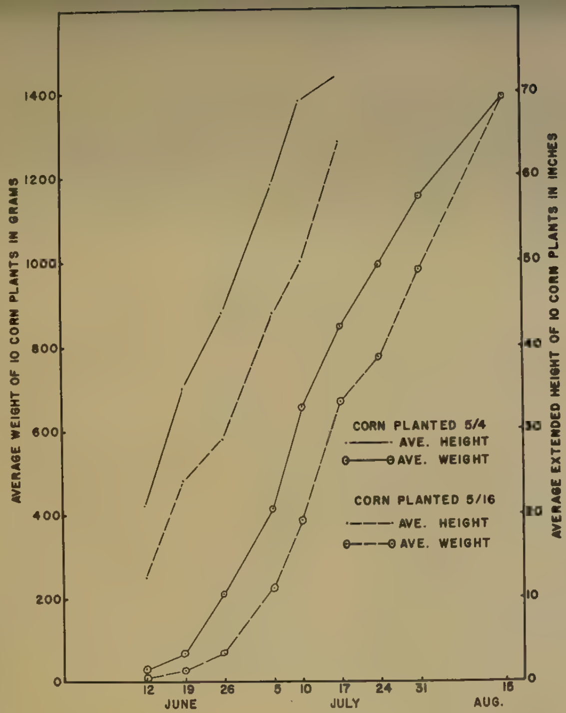


Fig. 13. Average height and weight of corn plants during 1951 growing season.

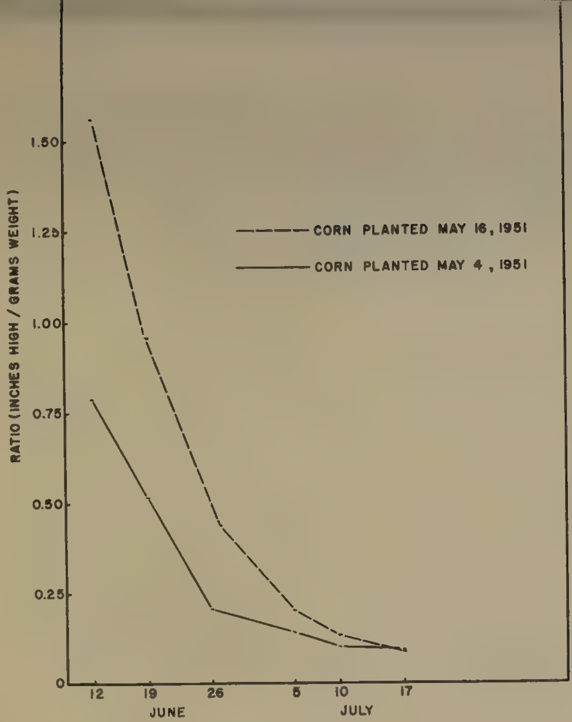


Fig. 14. Ratio of height to weight of corn plants during 1951 growing season.

TABLE 24

Effect of Plant Growth on Insecticide Residues on Corn, 1951

Application Date	Weight of One Plant Grams	Per Cent Increase in Plant Weight Between Spraying and Ensiling	Residue (p.p.m.) at Ensiling From a Deposit of 75 p.p.m.	Per Cent Loss in Residue Due to Plant Growth
June 12	26.8	5086	1.45	98.1
" 19	67.2	1968	3.63	95.2
" 26	209.0	565	11.29	85.9
July 5	417.0	233	22.52	70.0
" 10	659.0	111	35.59	52.5
" 17	842.0	65	45.47	39.4
Aug. 16*	1390.0	--	--	--

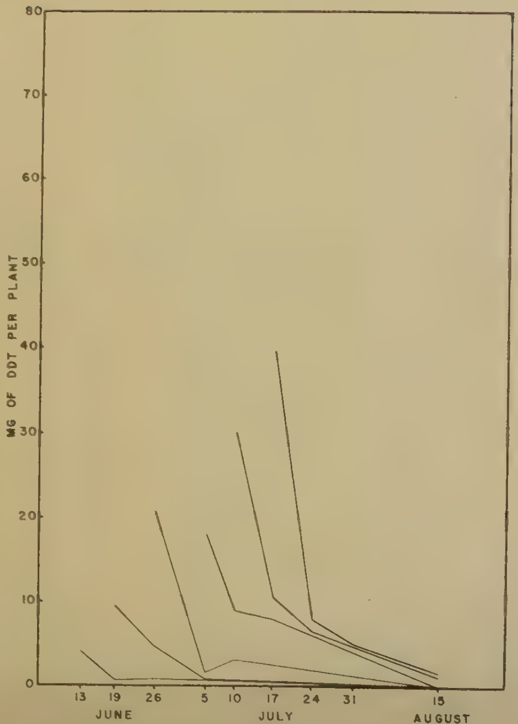
*Corn plants matured to ensilage stage.

TABLE 25

DDT Residues, in Milligrams Per Plant, From Single Application of Sprays on Corn Planted on May 4, 1951

Spray No.	Date of Sampling	Suspensions						Emulsions					
		No.1	No.2	No.3	No.4	No.5	No.6	No.1	No.2	No.3	No.4	No.5	No.6
1	June 12	--	--	--	--	--	--	-	--	--	--	--	--
	" 13	4.25	--	--	--	--	--	5.88	--	--	--	--	--
2	" 19	0.68	9.53	--	--	--	--	2.82	11.63	--	--	--	--
3	" 26	0.77	2.30	20.77	--	--	--	3.18	4.72	30.18	--	--	--
4	July 5	--	0.75	1.54	18.10	--	--	--	1.96	3.71	31.65	--	--
5	" 10	--	--	3.10	8.96	29.92	--	--	--	10.48	29.39	43.89	--
6	" 17	--	--	--	7.83	10.53	39.49	--	--	--	25.68	23.58	61.30
	" 24	--	--	--	--	6.57	8.17	--	--	--	--	16.63	17.93
	" 31	--	--	--	--	--	4.73	--	--	--	--	--	15.10
	Aug. 16*	0	0	0	0	1.11	1.67	0	0	1.81	8.62	9.17	7.51

*Corn plants matured to ensilage stage



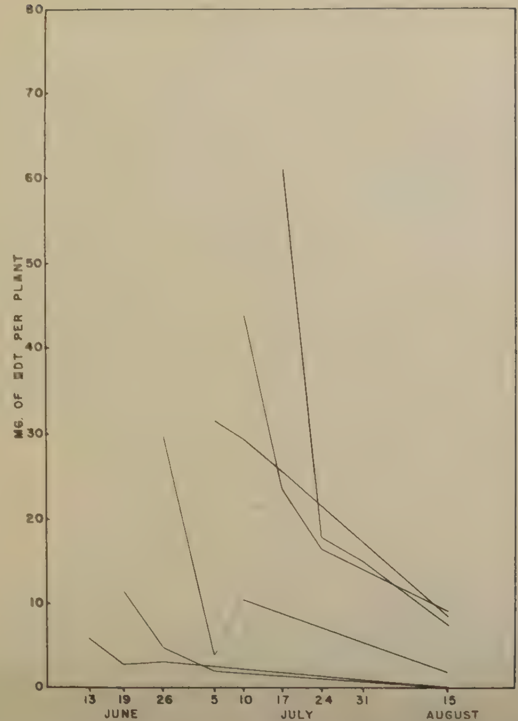


Fig. 15. Weathering of DDT residues from corn planted on May 4, 1951. Left, from suspension sprays; right, from emulsion sprays.

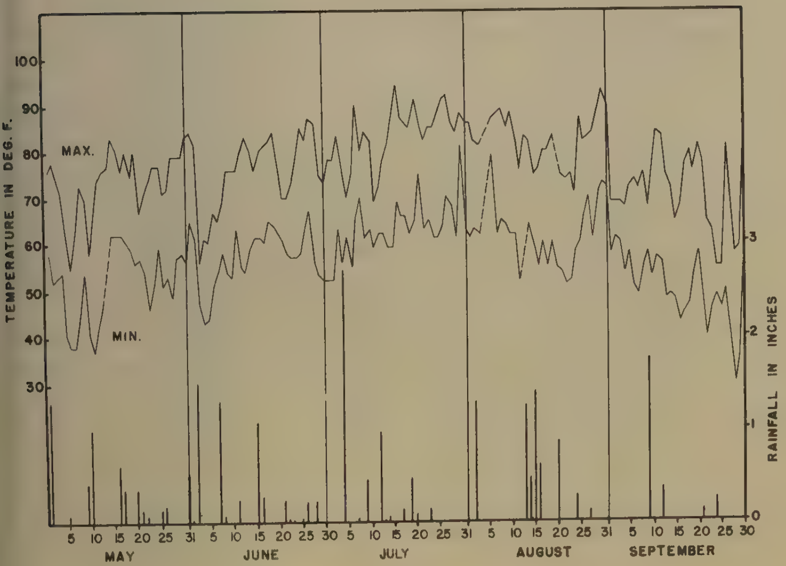


Fig.16. Temperature and rainfall data, May-September, 1951.

The residues from suspension sprays show a very rapid loss as a result of weathering, only 8 to 28 per cent of the original deposit remaining after 14 days. On the other hand, residues from emulsion sprays show some resistance to weathering, from 17 to 86 per cent of the deposit remaining after 14 days. With the suspension spray no residue remained at ensiling time on corn plants sprayed on June 5 or earlier, and the residue on plants sprayed July 17 was only 1.67 micrograms per plant. On the other hand, emulsion sprays applied between June 26 and July 17 left residues of 1.81 to 8.17 micrograms per plant at ensiling time. Thus the loss of deposit due to weathering varies considerably with the spray formulation.

Table 26 compares the loss of DDT from deposits of suspension and emulsion sprays due to weathering and shows the reduction in residues due to plant growth. In each comparison the deposit from the suspension spray shows a more rapid loss than the deposit from the emulsion spray. Except for the spray applied on July 5, 82 to 92 per cent of the DDT from suspension sprays and 46 to 83 per cent from emulsion sprays were lost during the first 14 days. In the reduction of deposits by weathering, time appears to be the most critical element. Plant growth is most effective in reducing deposits from early-season sprays, when the increase in weight is most rapid.

TABLE 26

Effect of Weathering and of Plant Growth Upon Reduction in Deposits from Suspension and Emulsion Sprays of DDT, 1951

Spray Date	Per Cent Loss of DDT				Per Cent Reduction of Deposit Due to Plant Growth	
	Suspension Spray		Emulsion Spray			
	After 14 Days	By Aug.16	After 14 Days	By Aug.16	After 14 Days	By Aug.16
June 13	82.4	100.0	45.9	100.0	87.2	98.1
" 19	92.1	100.0	83.1	100.0	83.9	95.2
" 26	85.1	100.0	65.3	94.0	68.3	85.9
July 5	56.7	100.0	23.2	72.8	50.5	70.0
" 10	79.6	96.3	62.1	79.1	33.8	52.5
" 17	88.0	95.7	75.4	87.7	27.0	39.4

Table 27 shows the initial deposits and final residues from sprays applied in 1951. Assuming that any residue greater than a trace (0.1 p.p.m.) would be objectionable in cattle feed, we can, with certain limitations, define the conditions under which residues can be avoided. If a single application of suspension spray is made before the corn is 60 inches in extended height, it will not leave undesirable DDT residues. When two applications are made, the final applications should be made before the corn is 44 inches high.

Residues from emulsion sprays are more persistent. A single application after the corn is 44 inches high will leave objectionable residues on the corn plants at ensiling time. If two applications are made a week apart, the second should be made before the corn is 35 inches high. Since this is contrary to recommended control practices, it seems impossible to avoid undesirable residues on corn for ensiling if emulsions are used.

STUDY OF DDT RESIDUES IN CORN KERNELS

Some organic insecticides are translocated in plant tissue. DDT is soluble in most vegetable and animal fats, and since corn kernels contain from 3 to 5 per cent of oil, the storage of DDT in the kernels is a possibility.

An experiment was so designed to show whether DDT applied to corn plants during the growing season would contaminate kernels produced by these plants. Two fields of corn, planted May 4 and 16, where employed. In each field three plots were sprayed with emulsion and three with suspension sprays. Two applications were made at 1-week intervals--on June 12 and 19, June 26 and July 5, and July 10 and 17. Table 28 shows the size of the corn plants at time of application as well as the deposit of DDT on the plants following the last spray.

TABLE 27

Effect of Date of Spray Application on DDT Residues on Corn at Ensiling Time, 1951

Date of Application	Corn Plant		P.p.m. of DDT							
			Suspension Spray				Emulsion Spray			
			One Application		Two Applications		One Application		Two Applications	
	Height Inches	Weight Grams	Initial	Final	Initial	Final	Initial	Final	Initial	Final
May 4 Planting										
June 12	21	26.8	158.7	0	--	--	219.3	0	--	--
" 19	35	67.2	141.8	0	142.3	0	173.0	0	243.3	0
" 26	44	209.0	99.4	0	162.6	Trace	144.4	1.3	171.1	2.0
July 5	59	417.0	43.4	Trace	66.8	1.5	75.9	6.2	78.5	7.2
" 10	69	659.0	45.4	0.8	55.9	1.6	66.6	6.6	96.0	7.3
" 17	72	842.0	46.9	1.2	56.9	2.6	72.8	5.4	93.2	12.4
May 16 Planting										
June 12	12	8.1	147.1	0	--	--	288.4	0	--	--
" 19	24	25.0	135.5	0	139.7	Trace	233.5	0	254.5	0
" 26	29	65.3	164.3	0	156.6	Trace	271.3	0	287.3	0
July 5	44	222.0	85.3	Trace	87.6	Trace	83.5	2.4	124.9	2.0
" 10	50	382.0	66.3	Trace	76.4	0.3	120.4	3.4	163.6	6.5
" 17	64	664.0	77.6	0.2	61.4	0.5	93.9	3.2	123.3	8.4

TABLE 28

Plots in Corn Fields Used in Study of DDT in Kernels, 1951

Plot No.	Height of Corn Plant, Inches At First Application	Height of Corn Plant, Inches At Second Application	P.p.m. of DDT On Plants After Second Application
	Field Planted May 4		
Suspension			
1	21	35	142.3
2	44	59	66.8
3	69	72	96.9
Emulsion			
4	21	35	243.3
5	44	59	78.5
6	69	72	93.2
	Field Planted May 16		
Suspension			
7	12.5	24	139.7
8	29	44	87.6
9	50	64	61.4
Emulsion			
10	12.5	24	254.5
11	29	44	124.9
12	50	64	123.3

Corn ears were harvested from these plots on October 23 for a study of DDT in the kernels. Two ears were selected from each of four plots in the following manner: The person taking the sample stood between the second and third rows of a four-row replicate and walked in 20 paces, then took the nearest ear on his right side (second row). He then made 10 additional paces and took the nearest ear on his left side (third row). He removed the outer husk with his left hand, keeping his right hand free from contact with the DDT-contaminated corn plants, and then with his right hand removed the ear from the plant and deposited it in a clean, new paper bag. Every effort was made to keep the corn ear free from contamination of residue from the corn plant. The corn in the paper bags was taken to the laboratory at Vincennes, allowed to air dry, and then shelled from the ear.

Attempts to analyze corn kernels for trace quantities of DDT (less than 1 p.p.m.) in previous seasons had met with little success, because of the presence of extractives in the residue solution that were not removed by the normal methods of purification, such as by washing with a

ixture of concentrated and fuming sulfuric acid or by chromatographic paration. A combination of these two procedures removed practically l interference from corn extracts. The technique employed follows:

One hundred and twenty grams of ground corn was placed in an 80-nce bottle, 240 ml. of colorimetric grade pentane was added and the ottle was sealed with aluminum foil. The bottle was tumbled for 3 ours, and the corn was then allowed to steep in the pentane for 48 hours. he pentane was filtered from the corn, and a 150-ml. aliquot transferred a clean beaker containing 30 grams of white calcined infusorial earth npregnated with 18 ml. of equal parts of concentrated and fuming (15 er cent SO_3) sulfuric acid. The samples were slurried with this mixture r 20 minutes, and then the pentane solution was decanted into a 500-ml. tandard-taper Erlenmeyer flask. The sulfuric acid-earth mixture was ashed with three 60-ml. volumes of pentane, and the washings were dded to the sample. The pentane was distilled to a low volume (about 10 nl.) in a hot-water bath and transferred to a chromatographic column ontaining 10 grams of Florosil (60/100 mesh) (in a 50-ml. burette) wetted ith pentane. The sample was then eluted with 200 ml. of pentane. The luate was evaporated to near dryness and transferred to a reaction tube, n which the drying was completed. The residue was analyzed by the tiff-Castillo method.

The treatment removed all interference except a slight opalescence hich activated the galvanometer needle of the spectrophotometer only lightly. Analyses of two lots of untreated corn to which 150 micrograms f DDT had been added gave photometer readings identical to that of 150 nicrograms of DDT added to the reaction flask. When the blank of 2.8 nicrograms (reading of untreated corn without added DDT) is taken into onsideration, this is a recovery of 98.1 per cent.

To determine the surface residue on corn kernels, 250 grams of corn vas placed in a funnel, washed with 100 ml. of chloroform, and rinsed wice with 50 ml. of chloroform. The washing and rinses were combined, and the chloroform was removed in a hot-water bath. The residue was analyzed for DDT by the Stiff-Castillo method. No evidence of DDT was found on the surface of any of the kernels of corn from the experimental plots.

To determine whether DDT was stored in the kernels, samples from each plot were washed in chloroform, dried, and ground in a burr mill. The corn was then analyzed by the technique outlined above. Two untreated lots of corn (one from each planting date) were included in the analysis. Although from 61.4 to 254.5 p.p.m. of DDT were found on the corn plants following spray applications, no DDT was found in the kernels.

SUMMARY

In 1949, 1950, and 1951 studies of the magnitude of DDT residues on corn plants were conducted by the Bureau of Entomology and Plant Quarantine in cooperation with the Iowa Agricultural Experiment Station. In all these studies DDT was applied at the rate of 1.5 pounds per acre in sprays and at 2 pounds per acre in dusts.

Investigations of the effect of spray volume, spray pressure, and nozzle arrangement on the deposition and distribution of DDT on corn plants

were conducted in 1950 and 1951. Sprays were applied at various dilu tions from 2.5 to 80 gallons per acre. The maximum deposits wer obtained at 2.5 gallons per acre, an indication that, at this volume, ther is not sufficient moisture to wet any part of the plant to run off. At 2 and 40 gallons per acre, there was approximately the same distributic of DDT on the plant parts and indication that the entire plant was thorough ly wetted with spray. At 80 gallons per acre there was excessive wettir of the plants.

At the minimum pressure employed, 20 pounds per square inch, resi dues were greater than at higher pressures. However, the difference were not great, and it is improbable that they would have caused signifi cant differences in insect control.

In a comparison of nozzle arrangements, 80-degree flat-fan nozzle (single or two in tandem) deposited appreciably more insecticide in th plant whorl than the arrangement of two or three 65-degree flat-fan noz zles over the row. Single hollow-cone nozzles deposited less insecticic in the whorl than either the 65- or 80-degree flat-fan nozzles. The Iow State nozzle arrangement deposited less insecticide in the whorl than wa obtained when all the spray was applied from nozzles over the corn row With all nozzle arrangements the residues found on leaves were heav and there was no appreciable differences in the residues found on th plant stalk.

Investigation of the magnitude and persistence of DDT deposits fro three formulations, dusts, wettable powders, and emulsifiable concer trates, the last two applied as sprays, showed: (1) that dusts gave low deposits than sprays and that the deposits from emulsion sprays wer greater than those from suspension sprays. The deposits from emulsic sprays were the most persistent. Aerial application of an emulsion spra resulted in lower deposits than ground applications of a similar spray The aerial application of a dust resulted in an uneven distribution of de posit, with excessive deposits on the middle rows and little or no depos on the outer rows.

When dust applications were made at night, with dew on the plants, th deposits were increased over those obtained when the dusts were applie during the day on dry plants. Deposits from sprays did not show any di ference between day and night applications.

When used for control of second-generation European corn borer (Pyrausta nubilalis (Hbn.)) on canning corn, three dust applications le residues of approximately 13 p.p.m. at ear harvest, and spray applica tions left residues of 29 to 41 p.p.m. When corn plants carrying a res due of 41 p.p.m. were ensiled, the silage contained from 12 to 34 p.p.m of DDT. Milk from cows fed this silage contained from 0.6 to 3.8 p.p.m of DDT.

Young corn plants, in which the ratio of height to weight (inches pe gram) is high, retained larger residues than older corn plants in whic the ratio of height to weight is low and the plant surface less permeable The effect of plant-growth dilution was greater during the period of rap development than after the plants have attained most of their growth During the first 14 days' exposure to weathering, deposits from suspen sion sprays showed a greater loss of residue than deposits from emulsic sprays.

In order to avoid residues in excess of 0.1 p.p.m. of DDT on corn ants used for silage or stover feeding, a single suspension spray should applied before the plants reach a height of 60 inches, and if two appli-tions are made the second should be made before the plants are 44 ches high. Single applications of emulsion sprays can be made before e plants are 44 inches high, or two applications a week apart before the ants are 35 inches high.

Corn kernels, collected at harvest time from plots that had been rayed twice with DDT at the rate of 1.5 pounds per acre when the plants ere 12.5 to 72 inches high, contained no DDT either on the surface or ithin the kernels.

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STUDIES OF TEMPERATURE AND HUMIDITY AT VARIOUS LEVELS IN CROP COVER WITH SPECIAL REFERENCE TO PLANT DISEASE DEVELOPMENT

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The temperature and humidity experienced by plant pathogens on plants n the field have been approximated by measurements obtained from the ir layers around plants. This has been necessary because of difficulty nvolved in making these measurements on the plant parts themselves. Iowever, recently, coincident air temperatures and plant part tempera-ures were measured with thermocouples (7). This investigation provid-d information basic to the prediction of plant temperatures from air emperatures. Unfortunately, instruments are not available to measure he humidity on plant part surfaces. Previous investigations of tempera-ure and humidity in different crops have shown that a vertical tempera-ure gradient exists between the soils and levels above (1, 2, 3, 4, 6, 7,). The immediate problem then is whether these gradients are of sufficient magnitude to influence the spore production and germinatinn of a plant pathogen such as Phytophthora infestans on potato and tomato. If so, measurements should be taken in plant cover under environmental conditions generally representative of those on plant parts.

Obtaining measurements of temperature and humidity in plant cover was suggested in 1946 as a result of an investigation of potato blight (5). The study revealed that differences in temperature between the potato cover and the level 5 feet above were slight but humidity differences ranged from about 2 to 14 percent depending upon the time of observation. The author suggested that the relative humidity measurements representtative of those experienced by the late blight pathogen should be taken in plant cover.

Another problem of immediate concern involves selection of the humidity expression that most closely approximates the conditions experienced by the phytopathogen at the leaf, stem or petiole surfaces and which expresses the moisture function most applieable to the problem under consideration. The expressions that could be employed are wet bulb

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temperature, dew point temperature, vapor pressure, vapor pressure deficit, relative humidity, absolute humidity, specific humidity and mixing ratio.

The present investigation was concerned with: (a) gradients of temperature and humidity obtained in a tomato and potato plot, (b) correlative observations of temperature and humidity obtained in corn, soybeans and over bare ground, and (c) comparisons of gradients determined from different expressions of atmospheric moisture.

MATERIALS AND METHODS

Bedford, Indiana.

In a 50 x 70 foot potato plot, wooden stakes were driven into the soi at two random locations. Nails located every foot served as hangers fo the hand aspirated psychrometer used in taking the wet and dry bulb temperatures. Two other stakes were located at random in an adjoining tomato plot of equal dimensions.

Psychrometric data were taken at 5 AM, 9 AM, 5 PM, and 9 PM ove a 22 day period during June and July. During the 9 AM and 5 PM readings the psychrometer was shaded. Because of the large amount of dat involved only 10 of the 22 days data were used in the following text.

Ames, Iowa

During an 8 day period, psychrometric data were taken at 4 AM an 4 PM at 1, 15, and 60 inches above the soil in corn, soybeans and ove bare ground. In all daytime observations the psychrometer was shade from direct radiation. Data on air temperature, dew point, vapor pres sure deficit, relative humidity and wet bulb temperature were submitte to variance analysis.

EXPERIMENTAL RESULTS

The means of the dry bulb temperatures, wet bulb temperatures, de point temperatures, vapor pressures, vapor pressure deficits and rela tive humidities were summarized (Table 1) for a 10 day period in potat and tomato cover.

The analysis of variance for temperature and relative humidity (Tabl 2) showed highly significant differences for days, times and levels for dr bulb temperatures and relative humidities. Although statistically signi ficant, the maximum difference between levels was 1.1°F.

No significant differences in relative humidity were found between th drops. This might be expected because of the similarity between potat and tomato cover when interplanted.

The other atmospheric moisture data shown in Table 1 were not sub jected to statistical analysis. However, the magnitude of the various ex pressions of atmospheric moisture was interesting, i. e. the relativ humidity became lower during the midday hours but the values of the othe expressions became higher.

Psychrometric readings indicated that saturation was approached ea lier in the evening in the atmosphere in the plant cover than at the 4- an 5- foot levels. The coincidental observation that dew deposition or fre water occurred first on the top leaves may be explained by the fact tha

Table 1.

Mean Dry Bulb Temperature, Wet Bulb Temperature, Dew Point, Vapor Pressure, Vapor Pressure Deficit and Relative Humidity taken at Four Times at Five Levels in Tomato and Potato Cover over a Ten Day Period.

Bedford, Indiana

Hour	Level In Ft.	Dry Bulb Temperature °F.		Wet Bulb Temperature °F.		Relative Humidity %		Vapor Pressure Deficit in		Vapor Pressure in		Dew Point Temperature °F.	
		Tomato	Potato	Tomato	Potato	Tomato	Potato	Tomato	Potato	Tomato	Potato	Tomato	Potato
5 A.M.	1	66	66	65	65	95	93	.043	.043	.595	.595	64	64
	2	66	66	65	65	94	91	.043	.043	.595	.595	64	64
	3	66	67	65	65	92	90	.043	.066	.595	.595	64	64
	4	66	67	65	65	92	89	.043	.066	.595	.595	64	64
	5	67	67	65	65	91	89	.066	.066	.595	.595	64	64
9 A.M.	1	81	81	72	72	66	66	.372	.372	.684	.684	68	68
	2	81	81	72	72	64	66	.372	.372	.684	.684	68	68
	3	81	81	72	71	64	63	.372	.418	.684	.638	68	66
	4	81	81	72	71	62	62	.372	.418	.684	.638	68	66
	5	81	81	71	71	60	61	.453	.418	.638	.638	66	66
5 P.M.	1	80	80	73	73	70	72	.290	.290	.732	.732	70	70
	2	81	80	72	73	67	72	.372	.290	.684	.732	68	70
	3	81	80	72	72	65	70	.372	.315	.684	.707	68	69
	4	81	80	72	72	65	69	.372	.315	.684	.707	68	69
	5	82	81	72	72	64	68	.407	.372	.684	.684	68	68
9 P.M.	1	69	69	68	68	96	96	.023	.023	.684	.684	68	68
	2	70	69	68	68	95	95	.071	.023	.661	.684	67	68
	3	70	69	68	68	93	93	.071	.023	.661	.684	67	68
	4	70	70	68	68	91	92	.071	.071	.661	.661	67	67
	5	71	70	68	68	90	91	.096	.071	.661	.661	67	67

Table 2.

Variance Analyses of Temperature and Relative Humidity Data Taken Over Ten Days in Two Crops at Five Levels at Four Times

	Dry-bulb Temperature				Relative Humidity			
	D.F	S.S.	M.S.	F-value	D.F	S.S.	M.S.	F-value
Day	9	1635	181.66		9	11400	1266.6	
Crop	1	5	5.05	2.68	1	32	32.0	.88
Level	4	45	11.25	18.44**	4	1294	323.5	41.47**
Time	3	16992	5664.0	79.45**	3	71540	23846	57.4 **
Crop x day	9	17	1.88		9	326	36.2	
Day x level	36	22	.61		36	281	7.8	
Time x day	27	1925	71.29		27	11212	415.25	
Level x crop	4	2	0.5	2.0	4	9	2.25	0.56
Level x crop x day	36	9	.25		36	144	4.0	
Time x crop	3	20	6.66	1.74	3	603	201.0	2.40
Time x crop x day	27	103	3.81		27	2261	83.7	
Time x level	12	8	.66	1.78	12	30	2.5	0.44
Time x level x day	108	40	.37		108	609	5.63	
Time x crop x level	12	0	0	0	12	45	3.75	0.87
Time x crop x level x day	108	16	0.15		108	464	4.29	

there was greater net outward radiation from top leaves. For some time before sunset, outgoing exceeds incoming radiation (2). Therefore the top leaves cool first to the dew point.

During the period of psychrometric observations, the development of *Phytophthora infestans* from 9 inoculated plants was observed in the plot. The results published elsewhere (8) indicated that nocturnal humidities and temperatures were the most critical to the development of P. infestans. However, during the night, the temperature and humidity differences between levels were insignificant. Therefore, meterological data may as well be taken at the 5-foot level as in plant cover.

Correlative observations of temperature and humidity in corn, soybeans and over bare ground were made over an 8 day period at Ames, Iowa. The averages shown in Table 3 indicated that with the exception of the 4 AM observations in corn, the temperatures were cooler at the lower levels. Moreover, in the 3 types of cover, atmospheric humidity was higher at the lower levels.

Temperature differences between levels were small in any of the 3 types of cover. At 4 AM the greatest temperature difference between levels was 2.3° F. over bare ground. Humidity differences between levels were small also at 4 AM but at 4 PM differences of 7 per cent were measured between levels in corn. At 4 AM relative humidity differences were small between crops, but at 4 PM differences up to 16 per cent were observed.

The analysis of variance showed that of the atmospheric moisture expressions used all were significant at 1600 for levels and crops. At 0400 vapor pressure deficit and relative humidity were not significant for levels and crops, while dew point and wet bulb temperature were significant for crops but not for levels.

The data indicate that at 4 AM measurements taken at the standard 5-foot level satisfactorily represented conditions in that crop cover. Differences between crop covers, although statistically significant in some cases, were probably of little consequence to the success or failure of the development of a phytopathogen in the foliage. At 4 PM humidity measurements should be taken in the crop and at the height desired. In most instances, the level of humidity would be too low to affect the reproductive and infection processes on warm dry summer days of the fungi and bacteria attacking corn or soybean foliage.

SUMMARY

In general, over a 10-day period at Bedford, Indiana, the temperatures 75° F. or below and relative humidities 90 per cent or above which are essential to the reproduction and infection processes of Phytophthora infestans occurred between 6 PM and 6 AM. Nocturnal temperatures and humidities observed at all levels were favorable for the processes abovementioned. Therefore, during these critical hours, measurements may as well be obtained at the 5-foot level as in plant cover.

Air temperatures showed statistically significant differences between levels in potato and tomato cover. However, the greatest temperature difference between the one and five foot level means was 2° F., too small to be of much biological significance.

Table 3.

Air Temperature, Relative Humidity, Dew Points, Wet Bulb Temperatures and Vapor Pressure Deficit Obtained in Corn, Soybeans and Bare Ground at Three Levels. Ames, Iowa

Time	Level above ground in inches	Dry bulb temperature °F.			Wet bulb temperature °F.			Relative humidity percent			Dew point temperature °F.			Vapor pressure deficit in inches		
		Corn	Soy-bean	Bare ground	Corn	Soy-bean	Bare ground	Corn	Soy-bean	Bare ground	Corn	Soy-bean	Bare ground	Corn	Soy-bean	Bare ground
4 A.M.	1	62.6	61.7	62.0	62.3	61.1	61.3	97.8	96.7	96.4	60.4	61.8	61.1	0.012	0.023	0.023
	15	62.4	61.4	61.6	61.8	60.7	60.8	97.0	96.0	95.7	61.3	60.0	60.1	0.017	0.022	0.024
	60	62.1	62.2	62.6	61.5	61.4	61.5	96.4	95.1	94.4	60.8	60.7	60.7	0.020	0.028	0.033
4 P.M.	1	84.0	83.1	86.8	75.5	76.4	74.8	67.7	73.6	57.6	73.8	71.8	69.7	0.378	0.304	0.549
	15	84.4	83.3	85.2	74.8	75.1	73.2	63.8	68.7	56.6	70.6	71.7	67.9	0.426	0.361	0.532
	60	84.6	84.1	84.5	74.1	72.6	71.4	60.7	57.4	52.4	69.5	67.1	64.8	0.464	0.506	0.568

Potato and tomato cover gave non-significant differences in relative ımidity. Humidity differences up to 6 per cent were measured between vels during the day.

Temperature differences between levels within corn, soybeans or over ıre ground were small. Differences in relative humidity up to 7 per cent ·tween levels within a crop and 16 per cent between crops were measured ıring the day.

Observations taken at 4 AM or 5 AM at the standard five foot level were ənerally representative of those in plant cover. Those taken during the ıy, especially in the afternoon, were not always representative of those ithin the crop cover.

When analyzed, different expressions of atmospheric moisture gave ifferent levels of statistical significance between crops and levels.

Dry and wet bulb temperatures were taken at five levels in potato and ɔmato cover at four times on ten days. In addition, psychrometric data ere taken at three levels at two times over bare ground and in corn and oybean cover over an eight-day period. From these data, the following umidity expressions were determined: vapor pressure, vapor pressure eficit, dew point and relative humidity.

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ERRATA
(To replace Table 1, p 263, Vol. 28.)

Table 1. Mean Dry Bulb Temperature, Wet Bulb Temperature, Dew Point, Vapor Pressure, Vapor Pressure Deficit and Relative Humidy Taken at Four Times at Five Levels in Tomato and Potato Cover over a Ten Day Period. Bedford, Indiana.

Hour	Level in Ft.	Dry bulb temperature °F		Wet bulb temperature °F		Relative humidity per cent		Vapor pressure deficit mm.		Vapor pressure mm.		Dew point temperature °F	
		Tomato	Potato	Tomato	Potato	Tomato	Potato	Tomato	Potato	Tomato	Potato	Tomato	Potato
	1	66	66	65	65	95	95	0.043	0.043	0.638	0.638	64	64
	2	66	66	65	65	95	95	0.043	0.043	0.638	0.638	64	64
5 A.M.	3	66	67	65	65	95	90	0.043	0.066	0.638	0.661	64	64
	4	66	67	65	65	95	90	0.043	0.066	0.638	0.661	64	64
	5	67	67	65	65	90	90	0.066	0.066	0.661	0.661	64	64
	1	81	81	72	72	65	65	0.372	0.372	1.056	1.056	68	68
	2	81	81	72	72	65	65	0.372	0.372	1.056	1.056	68	68
9 A.M.	3	81	81	72	71	65	62	0.372	0.418	1.056	1.056	68	66
	4	81	81	72	71	65	62	0.372	0.418	1.056	1.056	68	66
	5	81	81	71	71	62	62	0.418	0.418	1.056	1.056	66	66
	1	80	80	73	73	72	72	0.290	0.290	1.022	1.022	70	70
	2	81	80	72	73	65	72	0.372	0.290	1.056	1.022	68	70
5 P.M.	3	81	80	72	72	65	68	0.372	0.315	1.056	1.022	68	69
	4	81	80	72	72	65	68	0.372	0.315	1.056	1.022	68	69
	5	82	81	72	72	62	65	0.407	0.372	1.091	1.056	68	68
	1	69	69	68	68	95	95	0.023	0.023	0.707	0.707	68	68
	2	70	69	68	68	90	95	0.071	0.023	0.732	0.707	67	68
9 P.M.	3	70	69	68	68	90	95	0.071	0.023	0.732	0.707	67	68
	4	70	70	68	68	90	90	0.071	0.071	0.732	0.732	67	67
	5	71	70	68	68	86	90	0.096	0.071	0.757	0.732	67	67